

2140

PROCEEDINGS

OF THE

SOCIETY FOR

EXPERIMENTAL BIOLOGY AND MEDICINE

INCLUDING THE

PACIFIC COAST BRANCH, IOWA BRANCH,

WESTERN NEW YORK BRANCH AND

PEKING (CHINA) BRANCH.

22

NEW YORK

1924 1925

In order to avoid delay in delivery or loss of the PROCEEDINGS and to obviate extra postal charges, members are requested to send promptly to the Secretary any *change of address* or change of *institutional connection*.

PRESIDENT—Holmes C. Jackson, University and Bellevue Medical College.

VICE-PRESIDENT—James W. Jobling, Columbia University.

VICE-PRESIDENTS *ex officio*—Chairman Pacific Coast Branch, William Ophüls, Stanford University Medical College; Chairman Minnesota Branch, Frederick H. Scott; Chairman Western New York Branch, Sutherland Simpson, Cornell University; Chairman Peking Branch, John F. Kessel, Peking Union Medical College.

SECRETARY-TREASURER—A. J. Goldforb, College of the City of New York.

ADDITIONAL MEMBERS OF THE COUNCIL—Stanley R. Benedict, Cornell University, and Peyton Rous, Rockefeller Institute, New York City.

EDITORIAL COMMITTEE—Drs. Goldforb, Jackson, MacNeal, Pappenheimer, Sherman, Wallace.

MANAGING EDITOR—A. J. Goldforb, College of the City of New York.

Press of
THOMAS J. GRIFFITHS & SONS,
Corner of Liberty and Hotel Sts.,
Utica, N. Y.

CONTENTS

	Page
SCIENTIFIC PROCEEDINGS:	
One hundred and forty-first meeting, October, No. 1, Vol. XXII.....	1
One hundred and forty-second meeting, November, No. 2, Vol. XXII....	75
One hundred and forty-third meeting, December, No. 3, Vol. XXII.....	135
One hundred and forty-fourth meeting, January, No. 4, Vol. XXII.....	209
One hundred and forty-fifth meeting, February, No. 5, Vol. XXII.....	267
One hundred and forty-sixth meeting, March, No. 6, Vol. XXII.....	315
One hundred and forty-seventh meeting, April, No. 7, Vol. XXII.....	363
One hundred and forty-eighth meeting, May, No. 8, Vol. XXII.....	418
LIST OF MEETINGS	572
MEMBERS' LIST (Alphabetical)	574
MEMBERS' LIST (Institutional)	579
OFFICERS	594
AUTHORS' INDEX	595
SUBJECT INDEX	607



Digitized by the Internet Archive
in 2024

SCIENTIFIC PROCEEDINGS.

ABSTRACTS OF COMMUNICATIONS.

One Hundred and Forty-first Meeting.

*New York Post-Graduate Medical School, Wednesday, Oct. 15, 1924
at 8:30 P. M.*

President Jackson in the chair.

I (2524)

The Reticulo-endothelial system in relation to antibody formation.

By FREDERICK P. GAY, M.D., and ADA R. CLARK.

[From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.]

The general lines of inquiry to determine the seat of antibody formation have been to seek the tissue where a given antigen is fixed; to determine the locality where the corresponding antibody may first be detected; or to prevent the formation of antibody either by specific injury (benzol, x-ray) or by extirpation of an organ. It has also been claimed that antibodies may be produced in tissue culture. With the evidence obtained by these methods it has been variously concluded that the essential antibody formers are the leucocytes; the leucocyte forming organs, spleen, bone marrow and lymph nodes; the liver; and the capillary endothelium.

We have probably been led astray by the expectation that some particular organ like the spleen, or some strictly localized cell group, rather than a more universal tissue, is responsible. Many of the phenomena of general immunity can be better explained on the assumption that some widely distributed cell-type is the essential antibody producer. The condition of strictly local immunity

in various parts of the body, which we believe has now been proved to exist, can be understood only on such an assumption.

The reticulo-endothelial system of cells (Aschoff) fulfills this criterion of wide distribution throughout the body including as it does one of the constituent elements of connective tissue and the capillary and lymph space endothelium, and being related to adult endothelium and the monocytes of the blood. It includes, moreover, the most markedly phagocytic and resistant cells of the body; indeed its differentiation depends on its ability to take up rapidly and to retain particulate matter and colloidal substances. The undoubted importance of spleen, lymph nodes and liver in the disposal of foreign cells and in antibody-formation, that has already been mentioned, would depend on their content of elements that make up the reticulo-endothelial system; and the failure of removal of one of these organs (*e. g.* spleen) to prevent antibody formation entirely, would depend on the vicarious or increased functioning of other parts of the same system.

It has occurred to others recently, as well as to ourselves, that a crucial experiment to prove the importance of the reticulo-endothelium in antibody formation might be effected by "plugging" or "blocking" the vacuolar segregation apparatus of these cells (clasmatocytes and capillary endothelium) with some indifferent non-protein colloid, and then attempting antibody formation. There are recent experiments which show that one colloidal substance when taken up by the clasmatocytes prevents absorption of a second colloidal substance, thus indicating that the segregation apparatus in these cells is a single one. Therefore, if this reticulo-endothelial apparatus is the antibody producer, saturation with any indifferent colloid should prevent antibody formation.

Standenath¹ and Vanucci² have recently published some such experiments on antibody formation. Standenath, who tested precipitin formation in rabbits after the use of China ink, found it was increased rather than diminished. Vanucci, who used both carmine and Wasserblau dye, thought subsequent agglutinin formation was decreased. These experiments not only disagree but are each in themselves wholly inconclusive when viewed in detail from the extremely small number of animals involved, and

¹ Standenath, *Zeit für Immunitäts Firsch*, 1923, xxxviii, 19.

² Vanucci, *Lo Sperimentale*, 1924, lxxxviii, 23.

the relatively slight differences in antibody strength between treated and controls.

Our own experiments on the effect of vital staining on hemolysin and bacteriolysin production are unequivocal. Rabbits and rats were saturated with Trypan Blue for about two weeks and then given three injections of sheep blood cells on successive days. In rats tested five days after the blood injections, during which period the Trypan Blue injections were continued, the serum of control animals untreated with Trypan Blue but given similar injections of blood, gave hemolysis in dilutions of 1-2500 to 1-10,000, whereas in the Trypan Blue animals it was negative at 1-10 in two animals and positive at 1-160 only in two others. In rabbits the hemolysin formation was followed more fully and showed similar differences between control and Trypan Blue animals. In controls the hemolysin titer was 1-1666 on the average in nine days, whereas the maximum production, reached only on the fourteenth day, in Trypan Blue animals was only 1-140.

Similar results have been obtained in the formation of bacteriolysins and agglutinins to the Cholera Vibrio. Our results on precipitin formation to horse serum after vital staining are as yet variable. It is possible that another mechanism is involved in the formation of this antibody.

The question naturally arises as to whether our success in preventing antibody formation by the use of a colloidal dyestuff has not been due to the injury of all the cells in the body, rather than to the specific "plugging" of the cells responsible for antibody formation. In view of the apparent harmlessness of Trypan Blue we do not believe that this explanation can be invoked, but further controls are necessary to check this possibility.

2 (2525)

Observations on bacteria in films, and the surface tension factor in phagocytosis.

By STUART MUDD and EMILY B. H. MUDD.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

The behavior of small solid bodies in contact with two immiscible liquids has been much discussed, especially in connection with phagocytosis. Direct observation of interfacial phenomena is possible with bacteria or other particles in films viewed in the darkfield microscope. A small drop of each phase, one of them containing the suspended bacteria, is placed on a clean slide. A cover slip is laid on top. The boundary between the two liquids appears as a brilliant band and the bacteria as shining motes. Bacteria in two phase films of water with a variety of organic liquids have been studied.

Ordinary Gram-positive or Gram-negative bacteria have been trapped in the liquid-liquid interface of all preparations examined. The trapping mechanism is much stronger in preparations with high (*e. g.*, hydrocarbon-water) than with low (*e. g.*, alcohol-water) liquid-liquid interfacial tension. Bacteria in the interface exhibit characteristic gliding movements along the interface, moving in the boundary line from regions of low to regions of higher liquid-liquid interfacial tension. Escape from the interface is possible in a variety of ways, *e. g.*, by sticking to the glass slide or cover slip, by Brownian movement, strong action of the flagella, jolting by other bacterial clumps, or even by centrifugal force while streaming rapidly around a curve in the interface. The efficiency of the interfacial trapping mechanism may be decreased by adding substances, *e. g.*, Na-oleate, lowering the liquid-liquid tension.

Acid-fast bacteria behave entirely differently. They exhibit little or no stability in the interface and pass readily or even spontaneously into the organic phase.

To account for these phenomena it is necessary to consider three interfacial tensions, T_{ow} the liquid-liquid tension, T_{so} the solid-organic phase tension, and T_{sw} the solid-water tension. If neither solid-liquid tension is greater than the sum of the other

solid-liquid tension plus the liquid-liquid tension, the bacterium will be trapped in the interface. If either solid-liquid tension exceeds the sum of the other two tensions, one liquid will spread on and engulf the bacterium; *i. e.*, if $T_{sw} > T_{so} + T_{ow}$, the organic phase will spread on and engulf the bacterium. This is the condition with acid-fast organisms in a water-oleic acid interface. The tension between the bacterial fat and fatty acid envelope and the oleic acid is low, T_{ow} is not high, and T_{sw} is greater than their sum. The bacterium passes spontaneously into the organic phase.

Similarly if $T_{so} > T_{sw} + T_{ow}$, the bacterium would pass into the water phase spontaneously. Clearly with the ordinary bacteria, $T_{sw} < T_{so} + T_{ow}$ and $T_{so} < T_{sw} + T_{ow}$, the inequality being greater as T_{ow} is higher.

Earlier attempts to formulate the surface tension factor in phagocytosis have assumed that surface tension would tend to carry the bacterium completely into either cell or plasma.

Fenn¹ has indicated the correct expectation to be that surface tension would retain the bacterium in the interface unless the condition of complete spreading obtained. Our observations substantiate the formulation of Fenn. The details will appear in an early number of the *Journal of Experimental Medicine*.

3 (2526)

Antirachitic properties imparted to lettuce and to growing wheat by ultraviolet irradiation.

By ALFRED F. HESS and MILDRED WEINSTOCK.

[From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.]

Wheat which was grown in the dark (etiolated) was found to have no antirachitic potency. Wheat which was grown in the light and irradiated with mercury vapor lamp conferred protection when fed to rats (10 gm. daily).

The same difference in regard to protective action against rick-

¹ Fenn, W. O., *J. Gen. Physiol.*, 1922, iv, 373.

ets was observed in vegetables which were irradiated after they had been plucked. Green lettuce leaves from the market were of no value in preventing rickets, whereas after irradiation when fed in same amounts (10 gm.) they had become antirachitic.

Therefore, in the plucked as well as in the growing green vegetable, irradiation led to the formation of an antirachitic factor.

Irradiated Wheat and Lettuce.

Rat Wt. gm.	Rickets Diet	Substance Fed (10 gm.)	Histologic Result
40-64 40-50 44-70	Low Phosphorus No. 84	Wheat <i>Irrad.</i> 1 hr. 1 ft. while growing	No. R. " " " "
40-60 44-68 40-60	"	Wheat (etiolated)	Mod. R. " " " "
40-59 40-52 40-60 24-40	"	Green Lettuce (<i>irrad.</i>) 1 hr. 1 ft.	No. R. " " Very sl. No. R.
41-61 34-54 40-64	"	Green Lettuce (<i>non-irrad.</i>)	Marked R. " " " "
44-50 40-42	"	No Lettuce	Mod. R. " "

4 (2527)

Antirachitic properties imparted to inert fluids by ultraviolet irradiation.

By ALFRED F. HESS and MILDRED WEINSTOCK.

[From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.]

The fact that inert fluids can be activated and rendered antirachitic by means of irradiation was reported by us some months ago.¹

Steenbock has reported a similar result in relation to the production of a growth-promoting factor. He has also confirmed

¹ Hess, A. F., *Am. Ped. Soc.*, June 7, 1924. Proceed. in *Am. J. Dis. Childr.*, 1924, xxviii, 517.

our observations in regard to rickets, having been able by means of irradiation to render "fats" active in preventing this disorder.² In our experiments various inert fluids were irradiated with the mercury vapor lamp for one hour at a distance of one foot, in order to ascertain whether by this means they could be endowed with antirachitic potency. It was found that cotton seed oil and linseed oil could be rendered specifically active by this means. After these oils had been thus irradiated they were able to protect rats from rickets when 0.1 cc. daily was fed in addition to the standard rickets-producing dietary (No. 84). The irradiated oils were able to store this factor for a considerable period. These experiments demonstrate the possibility of producing an antirachitic factor in vitro.

5 (2528)

Food accessory substances in bacterial growth. I. The influence of initial hydrogen ion concentration of media on the growth promoting effect of tomato extract.

By GREGORY SHWARTZMAN, M. D., (Introduced by I. S. Kleiner).

[From the Laboratory of Bacteriology, New York Homeopathic Medical College and Flower Hospital, New York City.]

It seems from a review of the literature bearing on the question of the rôle of vitamins in bacterial growth that nobody has investigated the influence of Hydrogen ion concentration of media on the growth promoting effect of these factors. The purpose of the present work was to study this relationship.

As tomatoes have been shown by Thjötta and Avery¹ to contain food accessory substances for bacteria, the growth promoting effect of tomato extract adjusted to pH 7.0 upon the growth of *B. Shiga* in media of pH range 5.4-9.0 was investigated.

It was found² that this extract is not able to promote the growth of *B. Shiga* in broth at initial pH 5.2-6.2, that it has a moderate

² Steenbock, H., *Science*, Sept. 5, 1924, 224.

¹ Thjötta, Th., and Avery, O. Y., *Exp. Med.*, 1921, xxxiv, N-1.

² A detailed report will appear elsewhere.

growth accelerating effect on this microorganism at initial pH 6.6-7.8 and has the best effect when added to broth having a pH 8.2-8.6. Furthermore it may be pointed out that the best growth of *B. Shiga*, in the presence of tomato extract, occurs at a pH (8.2-8.6) which is by itself an unfavorable one for the multiplication of this microorganism in plain broth.

Before going any further with this investigation it was decided to determine the factor responsible for the growth promoting effect of tomato extract in the case of *B. Shiga*.

Thjötta and Avery,¹ who used the tomato extract for initiating the growth of Pfeiffer bacillus in blood free media, demonstrated that the poor nitrogen content of tomatoes cannot alone be responsible for the acceleration of bacterial growth.

It seemed necessary to determine whether the high sugar content might be the responsible factor, since it was found* that the extract used in these experiments contained 3.54 per cent of reducing substance expressed as glucose. But a quantitative titration of the growth promoting factor of this extract demonstrated that the latter had a pronounced effect when added in a dilution 1:100. Evidently, therefore, the growth promoting effect of tomato extract cannot be attributed to its sugar content since in a dilution of 1:100 there will be only 0.0354 per cent of sugar, or an amount that could not have by itself any appreciable effect on the growth of bacteria.

However, bone charcoal absorption experiments brought further information as regards the nature of the factor in question, as it can be definitely stated that the factor which shows the optimum growth promoting effect in *B. Shiga* cultures of pH 8.2-8.6 can be removed by bone charcoal.

To complete the experimental data, the influence of tomato extract on the growth of *B. Flexner*, *B. Abortus* (Bang), *Pneumococcus* type 2, *B. typhosus* and *streptococcus viridans* in media of various H-ion concentrations were studied.

These experiments show definitely that the tomato extract has no growth promoting effect in media of pH 5.2-6.2 and the best effect at pH 8.2-8.6.

There was a moderate growth promoting effect of this extract at the pH range 6.6-7.8 in case of *B. Flexner*, *B. typhosus* and

* I am indebted to Dr. Israel S. Kleiner for the quantitative estimation of sugar in tomato extract.

streptococcus viridans, while the growth of *Pneumococcus* type 2 started to show the acceleration only at pH 7.8 and that of *B. Abortus* at pH 7.0.

Since the observations described above demonstrate that the food accessory substances of tomato show their optimum growth accelerating effect on certain bacteria at Hydrogen ion concentrations which seem to be absolutely independent of the optimum pH for their growth in ordinary media, an explanation of this phenomenon was sought. See 18 (2541).

6 (2529)

The action of pepsin on insulin.

By ALBERT A. EPSTEIN.

[From the Laboratory of Physiological Chemistry, Pathological Department, Mt. Sinai Hospital, New York City.]

Because of the practical advantages that would accrue from the oral administration of insulin in the treatment of diabetes, an exact knowledge of the mode of action of the digestive ferments upon it would be valuable. The various attempts made to procure physiological effects from insulin given by mouth have so far proved futile, and this failure has been ascribed to the destructive action of the gastro-intestinal ferments upon it. Recorded studies on the action of the individual proteolytic ferments on insulin agree that they 'destroy' insulin by proteolysis. These investigations assume that insulin is a 'protein' body, and that the loss of its physiological potentialities is the result of cleavage.

Certain considerations prompted a reexamination of this matter. In work recently reported,^{1, 2} it was found that trypsin does not actually destroy insulin, but that it merely renders it inactive

¹ Epstein, Albert A., and Rosenthal, Nathan, *J. Am. Med. Assn.*, 1924, lxxxii, 1990.

² Epstein, Albert A., Rosenthal, Nathan, and collaborators, *Am. J. Phys.*, 1924, lxx, 225 (in press).

by combining with it. The reaction which takes place between the two substances is in the nature of a chemical combination similar to that now known to occur between trypsin and safranin.³ The experiments referred to give the conditions under which trypsin inactivates insulin. Briefly, this inactivation takes place instantly when the pH of the medium is to the alkaline side of 4.6. Inactivation of insulin does not occur if the pH is below 4.6. From then on, dissociation or reactivation of insulin can be brought about even after many hours of contact with trypsin, by adjusting the pH of the medium to a point below 4.6.

Similar studies were undertaken with pepsin and insulin. The method of preparation of pepsin was as follows: Pepsin (Fairchild) was extracted with 50 per cent alcohol; the extract filtered and treated with 8 volumes of pure acetone. The precipitate was dissolved in water and passed through a Berkefeld filter. The solution was kept in the ice-box under sterile precautions. Such a solution of pepsin remains active for a long time and thus has the advantage that the same preparation can be used in a large series of experiments.

Pepsin and insulin mixtures were acidified (pH below 3.0) and portions of the mixtures were injected at different intervals into suitable test animals. It was thus found that under the conditions described, inactivation of insulin takes place instantly. The amount of insulin contained in each portion of the mixture used for injection was at least 15 units and frequently much more. The results have been uniform in that none produced the physiological effects of insulin. The type of acid used for acidification is apparently of no great consequence. The addition of buffered solutions to the pepsin-insulin mixtures yields the same result. Neutralization or alkalinization of the mixture prevents the inactivation.

The brief contact necessary for the inactivation of the insulin suggests that the process underlying it is not one of proteolysis. This supposition is supported by the fact that liberation or reactivation of the insulin can be effected by neutralizing or alkalinizing the pepsin-insulin mixture even after contact under conditions favorable for digestion (thermostat at 37.5° C.) for as long a period as 4 days.

While the exact range of the pH necessary for the inactivation

³ Marston, Hedley R., *J. Biol. Chem.*, 1923, xvii, 851.

of insulin by pepsin and its reactivation has not as yet been determined, it may be stated that the inactivation of insulin by pepsin and its reactivation, occur under conditions diametrically opposite, as far as the pH is concerned, to those necessary for a similar action of trypsin on insulin.

SUMMARY.

1. Pepsin 'inactivates' insulin but does not digest it.
2. Liberation or dissociation of insulin from pepsin takes place, even after prolonged contact, at a properly adjusted pH.

7 (2530)

Diphtheria toxin-antitoxin titration by Ramon method for practical application.

By OLGA R. POVITZKY and EDWIN J. BANZHAF.

[*From the Bureau of Laboratories, Health Department, New York City.*]

The Ramon test was evolved from the works of Calmette and Massol,¹ who in 1909 applied the flocculation test for titration in vitro of antivenom serum against Cobra venom. Nicolle, Cesari and Debains² in 1919 applied the same principle for titration of diphtheria and tetanus toxin-antitoxin by the method of Ascoli.³ The reaction consisted in the formation of an opalescent ring in contact with a concentrated toxin and gelatinized antitoxin. Georgi⁴ added a suspension of cholesterolized heart extract to the mixtures of toxin-antitoxin to obtain flocculation.

Ramon⁵ in 1922 found that diphtheria and tetanus toxin and antitoxin alone, when mixed in certain proportions, will bring about flocculation. The mixtures with a deficiency or excess of either toxin or antitoxin will fail to flocculate. The first precipitate to appear in the mixtures Ramon calls the "precipitate indi-

¹ *Ann. de L'Inst. Pasteur*, 1909, xxiii, 155.

² *Compt. Rend. Acad. des. Sci.*, 1919, clxix, 1433.

³ *Berl. Tierärztl. Wochenschr.*, 1911, No. 22, 389.

⁴ *Medizinische Klinik*, 1920, xvi, 1053.

⁵ *Compt. Rend. Soc. de Biol.*, 1922, lxxxvi, 711.

cateur" and this corresponds to a nearly neutral mixture. From the latter the values of toxin or antitoxin are calculated.

Scholtz⁶ in 1923 found that it was not necessary to employ for this test the enormous quantities of toxin used by Ramon (20 cc. for each tube). He used instead 2 cc. of a stable titrated toxin and corresponding dilutions of serum. Glenny and Okell⁷ in 1924 also used from 2 to 5 cc. of a stable titrated toxin and minute quantities of undiluted serum. All workers agree that the Ramon titrations agree closely with guinea pig tests and are therefore applicable for practical routine estimation of values of toxin and antitoxin.

Povitzky and Banzhaf working on this test since early spring of 1924 arrived at the same conclusions. They found that the most reliable and constant results are obtained by the use of a potent stable toxin titrated for its L+ and flocculation values and undiluted serum or (if the latter is sufficiently strong) diluted 1:1, that is, with an equal amount of physiological salt solution. The amount of toxin was usually 2.5 cc. per tube. They also tried to work with only 1 cc. of toxin, diluting the serum correspondingly. The results, however, with a serum diluted 1:5 were variable (delayed) in comparison with parallel tests with undiluted serum and larger amounts of toxin (2.5 to 5 cc.). It is very important to read the results on the same day the tests are made, since the next day more than two tubes may show flocculation and it would be impossible to tell which tubes precipitated first. With a stronger toxin, however, (this toxin is about 4 L + per cc.) the dilutions of the serum need not be so high and the test should be perfectly workable with 1 cc. of toxin.

In testing the serum and plasmas of thirty-five horses at different times the above authors found only a very few discrepancies between the results of guinea pig tests and Ramon flocculation method.

⁶ *Centralbl. f. Bact. Orig.*, 1923, xci, 72.

⁷ *J. Path. and Bact.*, 1924, xxvii, No. 2, 187.

PRACTICAL DIPHTHERIA TOXIN-ANTITOXIN METHOD 13

Diphtheria Toxin-Antitoxin Titration by Ramon Tests.

Horse No.		Amts. of serum per 2.5 cc. toxin*				Time of flocculation	Units of flocculation	Units by guinea pig test
28	P	.08	.07	.06	.05	2.20 hrs.	400+	375-400
	dil. 1:1	—	—	+	(+)			
33	P	.07	.06(\)	.05	.04	2½ hrs.	350 about	350-375
	dil. 1:1	—	+	±	—			
34	P	.08	.07	.06(\)	.05	3¾ hrs.	175+	175-200
		—	—	+	±			
40	P	.06	.05	.045	.04	3¼ hrs.	175 about	200-210
		(+)	—	—	—			
42	P	.04	.035	.03	.025	3 hrs.	250+	325-350
		(+)	—	—	—			
43	P	.04	.035	.03	.025	2¾ hrs.	250+	275-290
		(+)	—	—	—			
44	P	.045(\)	.04	.035	.03	overnight	225+	300-310
		+	±	—	—			
45	P	.045	.04	.035	.03	3¾ hrs.	300—	275-280
		—	+	(+)	—			
46	P	.06	.05	.045	.04	5 hrs.	175+	200-210
		(+)	—	—	—			
49	P	.06	.05	.045	.04	4 hrs.	200+	200-220
		+	(+)	—	—			
60	P	.15	.13	.12(\)	.10	overnight	100 about	175
		—	—	+	+			
62	P	.05	.045	.04	—	2 hrs.	225+	225 about
		+	(+)	—	—			
63	S	.095	.09	.085	.08	overnight	100+	200 minus
		+	(+)	—	—			
66	P	.08	.06	.05	.045	1¾ hrs.	200	175-180
		—	+	(+)	—			
67	P	.15	.13	.12	.10	overnight	75	175
		(+)	—	—	—			
68	S	.09	.085	.08	—	4¼ hrs.	100+	200 much less
		(+)	—	—	—			
69	P	.04	.035	.03	.025	4¾ hrs.	250+	250-260
		(+)	—	—	—			
72	S	.06	.05	—	—	overnight	175	175-200
		(+)	—	—	—			
73	S	.125	.12(\)	.115	.10	4 hrs.	100—	100-110
		—	+	+	—			

P = Plasma. S = Serum.

* 1 cc. toxin = about 4 L+. The flocculating value of the indicating mixture contains 4 units of antitoxin per 1 cc. of toxin. Units of antitoxin per cc. can be calculated from amounts used for flocculation.

† Tubes placed in water bath at 50° C. Tubes not showing reaction on same day were placed in incubator overnight.

(+) show "indicating mixture". (\) between two numbers, take number between the two, or average number.

8 (2531)

The production of an exotoxin by certain strains of
staphylococcus aureus.

By JULIA T. PARKER.

[From the Department of Bacteriology and the Department of
Pathology, College of Physicians and Surgeons, Columbia
University, New York City.]

We have succeeded in demonstrating in sterile filtrates of broth cultures of four out of twenty-one strains of *staphylococcus aureus* isolated from various conditions, a powerful poison with a selective action for the skin. The poison is produced by the growth of these strains in any well buffered broth medium containing only a small amount of glucose. The medium from which our most powerful poisons were obtained was prepared by adding an equal volume of M/15 phosphate buffer solution pH 7.4 to ordinary sugar—free meat infusion broth containing 4 per cent Witte peptone, boiling, filtering and autoclaving. After inoculation with a suitable strain of staphylococcus, the poison can be demonstrated in the sterile Berkefeld filtrates of this broth after 24 hours growth, but our most toxic poisons were obtained after four to six days growth.

The toxicity of the filtrates was tested by intracutaneous inoculation in rabbits: One-tenth cc. of filtrates was inoculated intradermally and at the same time a control of 0.1 cc. of uninoculated broth was always injected in the same rabbit.

The reaction produced by the injection of a toxic filtrate becomes evident from two to six hours after injection as a bluish-purple area of 2 to 5 cm. in diameter, depending on the toxicity of the filtrate. The next day, the purple color assumes a yellow tinge, and there is usually added a deep red zone of 0.5 to 3 cm. surrounding the yellowish area. The circumscribed yellowish area of 3 to 5 cm. becomes progressively yellower—as if necrotic. By the fifth day brown patches appear in the yellow area and increase in size until, at about the twentieth day, the whole lesion has become a dark brown scab. Four to eight weeks later the scab falls off leaving an ulcer. Microscopically the lesions show marked infiltration of polymorphonuclear leucocytes with necrosis of epidermis and underlying corium.

The poison is extremely heat labile, being completely destroyed if heated to 55° C. for one hour. It can be preserved with only slight deterioration for at least two months, if kept in the dark at 4° C.

By intradermal injections of this poison in rabbits, we have been able to produce antiserums which neutralize it in vitro in multiple quantities, and, therefore, conclude that this dermatotoxic poison is a true soluble toxin. To date we have found only traces of antitoxin in the serum of rabbits injected intravenously with the toxin.

In a series of eighteen toxin-treated rabbits, it was found that the skin reactivity to the toxin of any one rabbit was inversely proportional to the amount of antitoxin which its serum contained. Normal rabbit serums only occasionally contain any demonstrable antitoxin, and then only in very slight amounts.

By neutralization experiments it has been shown that the toxins from our four active strains are identical.

Lesions microscopically similar to those caused by the toxic filtrates can be produced by intradermal inoculation with young broth or agar cultures.

The size of the lesions of the different strains so inoculated seems correlated with their ability to produce toxin in culture.

9 (2532)

The germicidal action of milk.

By JAMES M. SHERMAN and HAROLD R. CURRAN.

[*From Cornell University, Ithaca, N. Y.*]

The so called germicidal action of milk has given rise to much discussion and controversy as to its extent, significance and even its existence. All workers are in agreement that the germicidal effect, if such exists, is only slight in action and transitory in occurrence after the milk is drawn from the animal. Some doubt its existence at all; some believe it to be effective so far as some organisms are concerned but to have no effect upon the typical milk bacteria; others believe in a bactericidal action, but hold that it may occur in the milks of some cows and not in others.

and may or may not appear in the milk of the same cow at different times.

Studies made by measuring the rate of increase in the natural fortuitous flora of fresh milk have, quite obviously, given contradictory results. When fresh milk is inoculated with known pure cultures, the results are still not definite as the germicidal effect is here complicated by the presence of the natural lag period, in the culture used as the inoculum, before rapid growth ensues.

It seemed to us that the problem could be got at more directly, and more definite information obtained, by the application of modern knowledge of the bacterial growth curve. As is well known, the freshly inoculated bacterial culture passes through a latent or "lag" phase before multiplication begins. It is also well known that transplants taken from a culture only a few hours old, while it is in the rapidly growing stage, will show no lag but continue multiplication at the same rate when seeded in suitable media. We therefore inoculated freshly drawn aseptic milk with young cultures in the period of rapid growth. For this purpose *Strept. lactis*, probably the most perfectly adapted milk organism, was used. Samples of milk were brought to the laboratory immediately after being drawn and placed in a water bath at 37° C. They were then inoculated from rapidly growing cultures three hours old at 37° C.; sterile milk being used as the growth medium from which the inoculum was taken. In these experiments control tests were also run by inoculating at the same time the same culture into sterilized milk (autoclaved) and incubating as in the case of the raw fresh milk.

TABLE I.
The germicidal action of fresh milk upon *Streptococcus lactis*.

	Number of bacteria per cc.		
	Beginning	1/2 hr.	1 hr.
Autoclaved milk	9,560	20,600	59,600
Cow No. 1	18,300	19,500	28,000
Cow No. 2	10,600	15,500	30,000
Cow No. 3	9,760	13,000	19,600
Cow No. 4	30,260	29,900	33,600
Cow No. 5	9,500	7,900	30,300
Cow No. 6	48,600	65,500	75,300
Cow No. 7	57,600	89,600	98,200
Cow No. 8	9,000	15,100	32,600

As was to be expected, no lag occurred in the sterilized milk inoculated from the actively growing young cultures. In the case of the freshly drawn raw milks, on the other hand, a slight but definite inhibitory effect upon the growth of the organism was found. In most cases a lag period of about one half hour was thus induced in the young inoculum, after which rapid growth followed.

10 (2533)

A further note on regeneration of the cut spinal cord in fish.

By J. FRANK PEARCY and THEODORE KOPPÁNYI

(Introduced by A. J. Carlson).

[From the Hull Physiological Laboratory of the University of Chicago, Chicago, Ill.]

In 1922 Koppányi and Weiss¹ reported in a preliminary note regeneration of the spinal cord in the fish (*Carassius vulgaris*, a small specimen 4-5 inches), and in the larvae of the salamander (*Salamandra maculosa*). Their method was complete section of the vertebra and cord in the high thoracic region. In one experiment three vertebrae were removed with their corresponding spinal cord. Regeneration occurred in both cases. Regeneration was evidenced by functional return in paralyzed regions and morphologically by histological examination. After the section, the animals were quiescent caudad to the section. Three weeks later the salamander larvae showed motility of the posterior portion. The motility slowly recovered until four or five weeks after the section when there was apparently complete functional recovery; their locomotion was coordinate and regular. During this period some of the salamanders metamorphosed. This confirms Loeb's statement that transection of the spinal cord in Axolotl does not prevent metamorphosis. Regeneration was less rapid in the fish, requiring six to eight weeks. There was apparent functional recovery, for swimming was coordinate, but the animals always lay upon the side and seldom swam in the normal position. Histologically, in conjunction with Kolmer, we traced the fibers across the section. These fibers were somewhat fewer in number than in the normal cord. They were mostly gathered in bundles although there was considerable interlacing.

¹Koppányi, Th., and Weiss, P., *Akad. Anz.*, 1922, xii, 2.

In the salamander with the three vertebrae removed, the vertebrae and cord regenerated completely.

Our first experiments in the present series were done on the adult newts, *Diemyctylus viridescens* and *Diemyctylus torosus*. These animals were unsuitable for the experiments because the cord is so far dorsal that the body musculature is unable to hold the cut ends of the spinal cord in opposition when the vertebral column is transected. We used, therefore, the goldfish (*Carassius auratus*). These were large specimens from 8 to 14 inches in length. A small transverse section was made through the skin and the muscles spread apart and the entire vertebral column cut in two with scissors. There was no bony continuity remaining between the regions above and below the section. A probe was passed fully from side to side in the section. The spinal cord must have been sectioned. The skin was sutured with silk. Wound healing was slow and was incomplete in about six weeks time. During the sixth week occasional swimming movements of the fish behind the spinal transection were observed. These became more frequent in occurrence and more effective for locomotion so that after about two and a half months it had reached its maximum recovery. The animals lay constantly upon the side except when their swimming movements turned them into their normal position. They seemed to have very little power of orientation and when stimulated often showed circus movements. The swimming was rhythmic and coordinate. It could not be considered to be a Freusberg-like phenomena for the coordination between the parts anterior and posterior to the transection was definite and constant.

Thus functional recovery can be said to have taken place. Such a recovery may have resulted from a regeneration of axis cylinders from the nerve cells whose axones were cut. This would constitute a real morphological regeneration. There is another possibility, however. On the basis of Pütter's statements that cold blooded vertebrates have an indefinite period of growth, Carlson has suggested that undeveloped nerve cells in the cord above the section which have retained their embryonic potentialities, under the stimulus of the section grow peripherally, replacing the axones cut; thus there would be physiological recovery from morphological new growth instead of from morphological regeneration. The large number of nerve fibres crossing the

section, as recorded by Koppányi and Weiss, and the age of the animals favors the former explanation (morphological regeneration). Further work on the problem is contemplated.

We acknowledge with pleasure our thanks to Dr. Carlson for his encouragement and support, and to Mr. Parker and Mr. Young of the Lincoln Park Zoölogical Gardens, who kindly supplied us with the fish used in these experiments.

11 (2534)

The relative susceptibility to x-rays of the eggs and sperm of *Arbacia*.

By JAMES W. MAVOR and DAVID M. DE FOREST.

[From the Biological Laboratory, Union College, Schenectady, N. Y., and the Marine Biological Laboratory, Wood's Hole, Mass]

The eggs and sperm of the sea urchin, *Arbacia punctulata*, were exposed simultaneously to the same x-rayed treatment and subsequently the x-rayed eggs were fertilized by untreated sperm, and untreated eggs were fertilized by x-rayed sperm. Untreated eggs and sperm from the same sea urchins were used in the control cultures. Two sets of experiments have been completed, one during the summer of 1923 and the other during the summer of 1924. In 1924 the conditions of the x-ray treatment were so arranged that the temperature of the water in which the eggs or sperm were kept during treatment did not vary from that of the room in which all the cultures were kept by more than 1° C. The temperature of the room during the different experiments varied from 19° to 22° C. The x-ray treatment was given with a standard Coolidge tube, tungsten target, at 50,000 volts and 3 milliamperes, the distance from the target to the eggs and sperm being 25 cm. In 1923 a portable radiator type tube with tungsten target was used and run at 50,000 volts and 2.5 milliamperes. The distance from the target to the germ cells was 11 cm. and the glass cups were surrounded by a lead box covered on the top where the x-rays entered by a thin sheet of aluminum 3 mills in thickness.

After the treatment of the eggs and sperm, fertilization was brought about in finger bowls and the larvae were reared up to forty-eight hours after fertilization, at which time samples were fixed in 2 per cent formaldehyde. In these samples where development had proceeded at the normal rate in the controls it was found that a considerable number of the pleutei were in a stage where the larger arms were appreciably longer than the rest of the body. This stage was numbered six and the development previous to this was divided into five stages as follows: 1, blastula; 2, gastrula; 3, gastrula which has become triangular and shows the beginnings of the two larger arms; 4, these two arms have developed but are appreciably shorter than the rest of the body; 5, the arms are approximately the same length as the rest of the body. In the case of each sample the stage in development of one hundred larvae was recorded. Table I shows a typical count from an experiment. It will be noticed that the variation in the stage of development at the time of examination is greater in the case of the larvae developed from an x-rayed germ cell and greatest in those developed from an egg fertilized by an x-rayed sperm. The average given in the last column is obtained by multiplying the number of larvae in each stage by the number of the stage and dividing by the number of larvae observed, *i. e.*, 100. The intervals between the stages are, of course, not the same; nevertheless, the average obtained in this way gives an approximate measure of the stage of development of the group.

In Table II are given the results of some typical experiments at different doses. It is to be noticed (1) that all the doses used, even the smallest (3 M. A., 5 min. 25 cm.) caused a retardation in development as measured by the stage reached after forty-eight hours; (2) the amount of retardation increases with the dose and was greatest for the largest dose (2.5 M. A., 60 min., 11 cm.); (3) *for the same treatment the amount of retardation is always greater when the sperm are x-rayed than when the eggs are x-rayed.*

The experiments described were carried out at the Marine Biological Laboratory, Woods Hole, Mass., and the Biological Laboratory at Union College, Schenectady, N. Y., with the assistance of a grant from the American Association for the Advancement of Science.

TABLE I.
Experiment 16, Series of 1924.

Treatment before fertilization		Stage of larva after 48 hours.						
Eggs	Sperm	1	2	3	4	5	6	Average stage
control	control	—	—	—	—	10.6	89.4	5.89
x-rayed	control	—	—	.3	6.3	64.0	29.4	5.22
control	x-rayed	—	16.0	30.0	48.0	4.0	—	3.38

TABLE II.
Typical experiment selected from the two series of x-ray experiments.
milliamperes \times minutes
 $D = \frac{\text{milliamperes} \times \text{minutes}}{(\text{distance from target})^2}$

x-ray dose in "D"	Average stage after 48 hours		
	Control	Eggs x-rayed	Sperm x-rayed
2.5	5.43	5.11	5.02
10.	5.36	5.11	4.68
20.	5.24	4.15	3.51
62.	5.86	3.67	2.52
124.	5.99	3.59	2.07

12 (2535)

Conduction in the mammalian auricle as affected by changes in hydrogen ion concentration.

By E. COWLES ANDRUS* and A. N. DRURY**
(Introduced by Edmund P. Carter).

[From the Johns Hopkins Medical College, Baltimore, Md.]

The writers have performed a series of experiments upon the hearts of dogs perfused with oxygenated Locke's solution by a modified Langendorff method. Observations were made upon the effect of changes in (1) hydrogen ion concentration and (2) oxygen content of the perfusing fluid upon the transmission of the excitatory process in the auricle. Electrical records were obtained by placing paired non-polarizable electrodes upon the auricle in such a way that the conduction interval was recorded over a single stretch of 12-16 mm. or over consecutive portions

* Fellow in Medicine of the National Research Council.

** Working on behalf of the Medical Research Council.

of 6-8 mm each. Each pair of electrodes was connected with a galvanometer string.

The effects of changes in pH of the perfusate upon conduction in the auricle were consistent with those reported by Andrus and Carter¹ on the A-V conduction. An increase in the alkalinity of the perfusing fluid, pH 7.8, was attended by a faster rate of transmission both in the naturally beating and rhythmically driven heart. In the latter the same result was obtained whether the stimulating electrodes were placed upon the base of the auricle or upon the appendix. A pH lower than normal, pH 7.0, caused the excitatory process to be propagated more slowly, and again, this result was obtained in the auricles following the sinus rhythm or rhythmic induction shocks introduced through electrodes placed either upon the base or tip of the auricle. In the naturally beating heart an increase in rate followed change to a higher pH and a fall in rate a lower one. Both the development and propagation of the excitatory process were, then, affected by changes in reaction of the fluid bathing the tissue and in the same direction.

In certain experiments the perfusing solutions were saturated with nitrogen in place of oxygen with striking results. If the auricle was perfused with an oxygen-free solution of pH 7.0 there resulted almost immediately a profound reduction in the transmission rate. Soon there appeared a condition in which the transmission of the excitation became progressively slower the farther the wave traveled from its point of origin, so that records taken with three pairs of electrodes showed a longer transmission interval between the second and third than between the first and second. As this condition progressed the muscle under the distal contacts responded to only every second wave of excitation as recorded at the middle electrodes and finally ceased altogether to become excited. And finally there appeared a condition of 2:1 block between the proximal and middle contacts and of complete block between the middle and distal pairs. With the stimulating electrodes upon the tip of the appendix under similar conditions the same result was obtained, the tissue at the base of the auricle failing to respond while that of the appendix responded to each excitation. These effects disappeared upon return to a normal well oxygenated perfusate. As this progressive slowing of the

¹ Heart, 1924, ii, 97.

transmission rate became more pronounced the electrical complexes appeared more and more degraded.

With conditions of altered hydrogen ion concentration, then, the rate of propagation of the excitatory process in the auricular muscle undergoes definite changes, rising with increasing alkalinity and falling as the hydrogen ion concentration is increased. If, upon a state of increased hydrogen ion concentration there be superimposed one of absence of oxygen there results a condition in which the excitation wave moves slower the farther it travels and the electrical responses become progressively degraded until finally there may appear block within the auricular muscle itself. This condition bears a striking similarity to that found in nerve and described there as "decrement".

In a series of observations soon to be reported Drury has demonstrated progressive slowing of the propagation of the excitatory process beneath a pressure clamp.

13 (2536)

The sympathetic innervation of voluntary muscles.

By ALBERT KUNTZ and ALVER H. KERPER.

[From the Department of Anatomy, St. Louis University School of Medicine, St. Louis, Mo.]

The experimental anatomical studies of Boeke (1913),¹ Boeke and Dusser de Barenne (1919),² and Agduhr (1919)³ indicate clearly that, in addition to somatic afferent and efferent components of the cerebrospinal nerves, nerve-fibers of sympathetic origin also terminate on voluntary muscle-fibers. Not a few physiological studies tend to corroborate this anatomical finding, others yield only negative results. Consequently, the sympathetic innervation of voluntary muscles is not yet universally accepted even as an anatomical fact.

The material on which the present study is based was obtained from three dogs in which the somatic nerve-fibers supplying cer-

¹ *Anat. Anz. Bd.*, xliv.

² *Proc. Amsterdam Akad. von Wetenschappen*, xxi, 1227.

³ *Proc. Amsterdam Akad. von Wetenschappen*, xxi, 1231.

tain muscles had undergone degeneration following section of the corresponding nerve roots.⁴ In two of the dogs the roots of the seventh, eighth and ninth thoracic nerves were exposed by laminectomy and cut distal to the spinal ganglia and proximal to the communicating rami. These animals were killed four weeks later. To avoid confusion which might arise by reason of overlapping of the areas of distribution of the intercostal nerves or the plurisegmental innervation of muscle-fibers, muscle was taken for study only from the eighth intercostal space. Control material was taken from intercostal spaces in which the nerves were left intact. In the third dog the mandibular nerve was cut intracranially. When this dog was killed twenty-three days later, portions of the masseter and pterygoid muscles on the side of the operation and control material from the corresponding muscles on the opposite side were taken for study. In all cases muscle tissue in which the somatic nerve fibers had undergone degeneration and control material was prepared both by the gold chloride and the pyridine-silver method.

In the control material prepared by both methods the terminal branches of the nerves comprising both myelinated and unmyelinated fibers as well as the terminations of fibers of both types on muscle-fibers may be observed. Our observations on the end-plates of the ordinary myelinated somatic efferent fibers corroborate those of Boeke (1921),⁵ who described these end-plates as hypolemmal in position and resting on nucleated 'sole-plates' composed of granular sarcoplasm which lies superficial to the myofibrillae and in which is imbedded a delicate reticular structure. As the myelinated somatic efferent fibers approach the end-plate some of them are accompanied by a slender unmyelinated fiber which terminates in a small end-net or end-loop within the area occupied by the 'sole-plate' on which the motor end-plate rests. Doubtless, these are the accessory fibers described by Boeke. Similar slender unmyelinated nerve-fibers also terminate on muscle fibers entirely apart from the end-plates in which the large myelinated fibers terminate.

In the preparations of the intercostal muscles in which the somatic nerve-fibers had undergone degeneration, neither myelinated nerve-fibers nor their terminal structures are present. In some instances the area occupied by the 'sole-plate' is still appar-

⁴ We are indebted to Dr. F. J. Tainter for these operations.

⁵ Brain, xliv, 1.

ent. In the preparations of the muscles of mastication in which the fibers of the mandibular nerve have undergone degeneration, remnants of some of the end-plates of the myelinated fibers are still visible. In all these preparations slender unmyelinated nerve fibers, some of which are intimately associated with the blood vessels while others occur either singly or in small bundles entirely apart from the blood vessels, are present. None of these fibers, many of which terminate on muscle-fibers, show any evidence of degeneration. The terminal structures of these fibers, like the ordinary motor end-plates, are always hypolemmal in position and rest on a layer of granular sarcoplasm which lies superficial to the myofibrillae. Some of these fibers terminate in a single end-net or end-loop, others give rise to two or more terminal branches which terminate in smaller end-nets or end-loops of similar character. These observations conform in all essential details to those recorded by the authors named above regarding the unmyelinated nerve-fibers and their terminal structures in preparations of voluntary muscles in which the somatic nerve-fibers have undergone degeneration. Inasmuch as, in our experimental animals, all the somatic nerve-fibers supplying the muscles in question had been cut and ample time was allowed for the degeneration of these fibers we must conclude that the unmyelinated nerve-fibers which remain are sympathetic in origin.

14 (2537)

Experimental observations on the functional significance of the sympathetic innervation of voluntary muscles.

By ALBERT KUNTZ and ALVER H. KERPER.

[From the Department of Anatomy, St. Louis University School of Medicine, St. Louis, Mo.]

In this study we adopted the plan of extirpating the sympathetic trunk in the lumbar region on one side only, using dogs as the experimental animals, and comparing the tonus, the power of contraction and the resistance to fatigue of the muscles of both hind limbs both immediately after the operation (6 dogs) and after ample time (10 to 36 days) had been allowed for the degeneration of the sympathetic nerve-fibers supplying the mus-

cles of the hind limb on the side of the operation (4 dogs).¹ Only the more important results obtained can be reported in this paper.

In the animals which were allowed to live following the operation a degree of hypotonus of the muscles of the hind limb on the side of the operation could be demonstrated by palpation for some time, but gradually subsided. This is in accord with the observations of Negrin, Lopez and von Brücke (1917)² and Dusser de Barenne (1917)³ on cats. We are not prepared to state how long such hypotonus persists. In one animal it was still demonstrable two weeks after operation, in another it could not be demonstrated by palpation ten days after operation. However, when these dogs were placed under surgical anesthesia, even after the hypotonus referred to had apparently subsided, a marked difference in the flaccidity of the muscles of the two hind limbs became apparent. As the animals lay on their backs with the limbs free the hind limb on the side of the operation dropped to a lower position than the other. When equal weights of 100 or 200 grams were attached to both hind feet and suspended over pulleys at the end of the table the limb on the side of the operation was more fully extended than the other. Weights of 600 grams drew both limbs out equally, both being fully extended.

In order to secure graphic records of the contractions of individual muscles the hind limbs were fixed in moderate extension, as the animal lay on its back under surgical anesthesia. The tendons of the gastrocnemius muscles were isolated and cut at their insertions. A strong cord was attached to each isolated tendon and led over a pulley at the end of the table to an ergograph or to a muscle lever with a weight attached. The stimulating current was supplied by two or three dry cells and an inductorium. The stimulus was applied to the exposed sciatic nerves.

The most significant graphic records obtained are the curves of fatigue of the gastrocnemius muscles when the sciatic nerves were stimulated by means of a uniform tetanizing current. The muscle on the side on which the lumbar sympathetic trunk was extirpated invariably underwent fatigue in less time than the one on the opposite side. The initial contractions of both muscles, whether isometric or isotonic, were usually approximately equal.

¹ We are indebted to Dr. F. J. Tainter for these operations.

² *Pflüger's Archiv.*, Bd. 166, S. 55.

³ *Pflüger's Archiv.*, Bd. 166, S. 145.

When the ergograph was used the curve obtained on the side of the intact sympathetic trunk was maintained at a fairly constant level for some time, then dropped gradually to the base line. The curve obtained on the opposite side either began to drop at once or was maintained at a constant level but for a short time, then dropped to the base line rapidly. In those cases in which the experiments were made immediately after the extirpation of the lumbar sympathetic trunk the curve obtained on the side of the operation not infrequently reached the base line in less than half the time required on the opposite side. In those cases in which the experiments were made after the sympathetic fibers supplying the muscles of the hind limb had undergone degeneration the decrease in the resistance to fatigue of the gastrocnemius muscle in this limb was still more marked. Cutting of the sciatic nerve on the side of the operation had no further effect on the resistance to fatigue of the gastrocnemius muscle, under the conditions of these experiments. The curves obtained when the muscle lever with a constant weight attached was used indicate the same difference in the resistance to fatigue of the gastrocnemius muscles of the two limbs.

In order to obviate the possible effect of changes in circulation through the muscle due to the interference with the innervation of the blood vessels in the limb on the side of the operation, experiments were made under otherwise similar conditions but with the common iliac arteries ligated. It is probably safe to assume that while no blood is flowing through the common iliac arteries no considerable changes in the volume of blood flowing through the gastrocnemius muscles could be brought about by reason of their activity or the stimulation of the sciatic nerves. Yet the curves of fatigue obtained immediately after ligation of the common iliac arteries as well as those obtained after these arteries had been ligated 15 to 20 minutes show differences in the resistance to fatigue of the gastrocnemius muscles comparable with the differences apparent in the curves obtained before the arteries were ligated.

The positive results obtained in this experimental study following unilateral extirpation of the lumbar sympathetic trunk are: (1) a degree of hypotonus of the muscles of the hind limb on the side of the operation which gradually subsides, (2) marked flaccidity of the muscles of this limb as compared with the muscles of the other hind limb while the animal is under surgical

anesthesia, even after the initial hypotonus of these muscles has apparently subsided, (3) decreased resistance to fatigue of the muscles of this limb, as illustrated by the curves of fatigue of the gastrocnemius muscles when the sciatic nerves are stimulated by means of a tetanizing current.

These results seem to justify the conclusion that the sympathetic innervation is a factor in the maintenance of the tonus of voluntary muscles, although this need not be apparent in the normal activities of the animal. The fact that voluntary muscles in which the sympathetic nerve-fibers have undergone degeneration are more flaccid than the corresponding muscles on the opposite side while the animal is under surgical anesthesia seems to indicate that the tonus of voluntary muscle which is maintained through its sympathetic innervation, like the tonus of involuntary muscle, is less completely eliminated by anesthesia than the tonus which is maintained through its somatic innervation. The decreased resistance to fatigue of the muscles of the hind limb following extirpation of the corresponding lumbar sympathetic trunk, which is more marked after the sympathetic fibers have undergone degeneration than immediately following the operation, as illustrated by the curves of fatigue of the gastrocnemius muscles obtained in the above experiments, seems to indicate that the sympathetic innervation is an important factor in sustaining the activity of voluntary muscles under conditions of stress and that this involves an outflow of nerve impulses directly from the sympathetic ganglion cells. Doubtless, the sympathetic ganglion cells constitute a source of nerve impulses which are also an important factor in maintaining the normal efficiency of voluntary muscles.

15 (2538)

Sex-reversal following ovariectomy in the fowl.

By L. V. DOMM (Introduced by F. R. Lillie).

[*From the Department of Zoology, University of Chicago,
Chicago, Ill.*]

It has been known for a considerable period of time that the female in many breeds of birds takes on certain male characters following the removal of the ovary, either by ovariectomy or

pathological absorption. In the latter case the reduction of the ovarian mass may be only partial or for a short period of time, followed by recovery and regeneration of the ovary; or the ovary may be permanently reduced and destroyed. In all such cases the female assumes certain male characters, and the extent to which such male characters are developed and their permanency, apparently depends upon the degree of removal or reduction of the ovary.

It is the purpose of this paper to call attention to experiments being carried on in this laboratory which have a very special bearing on some of the work recently reported by Crew¹ in the fowl, and by Riddle² in the pigeon. Investigations along this line were suggested by Professor F. R. Lillie, early in the fall of 1922, and have been carried on under his direction.³

My work thus far has been confined exclusively to the Light Brown Leghorn race. Sexual dimorphism in this breed is very pronounced, and the birds are more active and perhaps somewhat more hardy than are the birds of some of the heavier breeds. Most of the birds were obtained from a reliable breeder immediately after hatching and reared in the laboratory, where operations could be made at various ages and results observed.

The operation in the female is relatively difficult, owing to the proximity of the great blood vessels. In the young female, prior to the age of laying, the ovary is a flat sheet of tissue attached intimately by one surface to the ventral surface of the left iliac and vena cava. It was found that the ovary is most readily and most satisfactorily removed during this stage of its development. If the operation is delayed until the changes preceding the laying season have set in, the ovary has enlarged and has become more extensively vascularized, and its removal is more difficult. As regards the operation, assuming that the preliminary steps have been accomplished and the ovary exposed to view, it was found very essential to carefully remove the dorsal peritoneum which covers the ovary. The ovary is now fully exposed and may be

¹ Crew, F. A. E. 1923. Studies in Intersexuality. II. Sex-reversal in the Fowl. *Proc. Roy. Soc., B*, Vol, 95, No. 667.

² Riddle, O. 1924. A case of complete Sex-reversal in the Adult Pigeon. *Am. Nat.*, Vol. 58, No. 655.

³ This work was aided by a grant from the Committee on Sex Research of the National Research Council.

removed by firmly applying a pair of forceps to the free borders and gently peeling it off. The initial attempts were almost invariably disappointing, owing to fatality or incomplete removal. However, a technique was developed by which the ovary could be completely removed without greatly inconveniencing the bird. After the ovarian mass had been carefully removed, all questionable fragments remaining were thoroughly seared with a hot scalpel. Aseptic methods were carefully followed, and both general and local anesthetics have been employed.

The results of complete castration in the female bird have not been so extensively recorded as the results following castration in the male, owing largely to the difficulties referred to. Some workers have even definitely stated that it is impossible to remove the ovary, that invariably fragments remain behind which regenerate in a short time, thus maintaining the normal ovarian influence. That the operation is exceedingly difficult, and that fragments may remain behind which will regenerate is not denied; but these obstacles may be overcome.

It was found by previous workers that, following the ablation of the ovary, the female takes on the male plumage at the following moult, the spurs begin to develop at once; comb, wattles, and earlobes become pale and small so that they approximate those of the capon in size and general appearance. These ovariectomized birds, though smaller, are very similar to the capon in appearance and are readily mistaken as such by those not knowing their history. While this type of transformation appears in our work it is the exception rather than the rule, and indeed is extremely rare as a definitive condition.

The larger percentage of our birds have assumed additional male characters following ovariectomy until they are practically complete replicas of the male, and, to those not familiar with their history, they are regarded as *unmistakable* males. Thus we find that they assume the complete male plumage, spurs grow as they do in the normal cock, head furnishings increase in size until they cannot be distinguished from those of the normal male. Other birds in the pen regard them as males and when a strange cock is introduced they fight as would other cocks, very frequently assuming the initiative, some of them having been observed to come off victorious in such a combat. Many of these birds crow regularly, and of these, some have been seen making

definite attempts at treading other hens, while one has actually been seen doing so. When aroused by a disturbance, it was found that their reaction is very similar to that of the male; the sound emitted, together with their reaction on such occasions, reminds one very much of the young male just prior to maturity. Some sixty birds have been ovariectomized, of which number approximately twelve are incompletely ovariectomized, while the majority of the others show the above changes in the male direction including the plumage, spurs, and head furnishings.

One set of experiments may be mentioned as an example: Out of one lot of fourteen females of the same hatch, one was kept as control and thirteen were ovariectomized between the ages of six weeks to six months; twelve of these have developed all the characteristics of the male mentioned above, some being completely cock-feathered, while the others are fast becoming so. The other one of the thirteen is very capon-like in appearance except perhaps for size and cannot be readily distinguished from her capon brothers by those not knowing her history. This bird has assumed complete male plumage, is developing spurs; but the comb, wattles, and earlobes are pale and small, resembling those of the capon.

A number of ovariectomized birds have been killed and examined. Bird No. 533 was hatched on April 26, 1923. On June 30, an ovariectomy was performed at which time the ovarian mass was apparently completely removed. The latter part of August it was noticed that the bird had become almost completely cock-feathered and that it was developing the comb and wattles of the male, in which respect it was difficult to distinguish it from its brothers. This development proceeded in the male direction until January 24, 1924, when the bird was killed and examined. At the time of killing, the bird was completely cock-feathered except for a few wing-coverts which were entirely hen-like, showing no modification in the male direction. It had the head furnishings of the male and had developed spurs. Its voice and reactions were decidedly male-like though it had never been observed to crow, but may have done so since no effort had been made to discover this reaction.

When the abdominal cavity was exposed we found on the left, corresponding in position to the normal ovary, a white testis-like organ, quite bulky, being somewhat longer than wide, and slightly

irregular owing perhaps to an encysted mass of cotton which had been left on the site of the removed ovary following the operation. Associated with this mass of testis-like regeneration was an ovarian follicle about the size of a dried pea. Histological examination revealed a tubular mass similar to the seminiferous tubules of the normal testis in the cock. The oviduct was present, though somewhat reduced.

On the right side, corresponding in position to the organ found on the left, was found a similar though larger testis-like organ measuring 2.3 cm. in length and 0.7 cm. in width; leading posteriorly from it was a small white strand resembling an immature vas deferens. The histological examination revealed a tubular mass with the typical structure of the normal immature testis.

Bird No. 543 was hatched on April 26, 1923, and was ovari-otomized on August 7, 1923. The bird was killed and examined on January 14, 1924. This bird was almost completely cock-feathered early in October, and it was noticed that the head furnishings were growing, being deep red in color, comparing favorably in size with those of its brothers though somewhat smaller. The bird at the time of killing was completely cock-feathered, head furnishings were of the male type and it had developed spurs. Its voice and behavior were typically male.

Dissection revealed on the site of the original ovary a testis-like organ similar to that found on the left in bird No. 533 and also associated with it was a small mass of ovarian tissue. The mass was slightly elongated, somewhat irregular and intimately attached by its outer margin to an encysted mass of cotton which had been left at the time of the operation. The ovarian part was found at the anterior outer margin and could be readily distinguished, consisting of a few follicles varying in size. The oviduct was present though considerably reduced. The histological examination revealed immature seminiferous tubules.

On the right side a testis-like organ was found corresponding in position to that on the left. This organ was somewhat larger, measuring 2.3 cm. in length and 0.8 cm. in width, and weighing 450.01 mg. Leading posteriorly from it was a small white strand resembling a vas deferens. This organ was not only larger than that found on the left, but was also much more regular in outline, giving the general appearance of a small immature testis. The histological findings proved it to be an immature testis with the characteristic tubular mass.

Bird No. 619 was hatched the early part of July, 1923, and ovariectomized on January 13, 1924. On May 29, 1924, the bird was killed and examined. At the time of killing the bird was not completely cock-feathered, probably less than half the feathers being cocky. Some of the feathers on the breast showed cocky tips and henney bases very distinctly, and some on the back and saddle, less distinctly. The bird had developed the head furnishings typical of the cock and was developing spurs.

Examination of the original ovarian site revealed no tissue which could possibly be ovarian or testicular, removal had been complete, and no regeneration had taken place. The oviduct was present and approximately normal in size.

On the right side, corresponding in position to the ovary on the left, a testis-like organ was found which was somewhat irregular in shape. The organ measured 2.3 cm. in length and 1.2 cm. in width. Leading posteriorly from it was a small white strand which could be followed to the cloaca and which presumably was an immature vas deferens. The organ has not yet been studied histologically.

The greater number of our ovariectomized birds show equally pronounced external male characteristics. They are being kept a time in order to see what the eventual transformation may be.

The results as here obtained appear to be relatively rare so far as the earlier investigations along these lines are concerned. Goodale⁴ was probably one of the earliest investigators who made extensive observations on completely ovariectomized fowl. Goodale observed a regenerated mass on the right side in some of his ovariectomized birds which resembled histologically early nephrogenous tissue. Pezard⁵ states definitely, in commenting on the work of Zawadowsky, that he never observed regeneration on the right side in any of his work. Zawadowsky⁶ observed regeneration on the right side and correlated with it was a more or less

⁴ Goodale, H. D. 1916. Gonadectomy in relation to the Secondary Sexual Characters of some Domestic Fowl. *Carnegie Inst. Wash. Pub.*, No. 243. Goodale, H. D. 1916. Further developments in Ovariectomized Fowl. *Biol. Bull.*, No. 30.

⁵ Pezard, A. 1922. Modifications periodiques ou definitives des caracteres sexuels secondaires chez les gallinaces. *Ann. des Sc. Nat. Zool.*, S. 10, V. 6.

⁶ Zawadowsky, M. 1922. Sex and Development of Sex Characters. Moscow, State edition, Russian with 20 pages of German summary.

complete assumption of male characters. Benoit⁷ reports similar results following ovariectomy.

In contrast to these observations several authors have reported the presence of glandular structures on the right side in birds showing pathological reduction of the ovary, and it is to this category that the cases of sex-reversal reported by Crew for the fowl, and Riddle for the pigeon, belong. Thus in such cases there has been found an ovotestis on the left associated with a testis-like structure on the right (Crew) or these structures may be unilateral, occurring only on one side. These birds very rarely show the complete secondary sexual characters of the male, but more frequently show maleness in only one or two of these characters; thus the bird may show only spurs of the male, or spurs and head furnishings may be male-like, while the plumage is henny, and correlated with these characters there is usually a certain degree of male behavior.

We thus find in the bird, as a result of our ovariectomy experiments, a condition analogous in many respects to that reported by a large number of authors in cases of pathological absorption of the ovary. There is a clear possibility that these ovariectomized individuals may undergo complete sex-reversal. They are certainly more complete replicas of the male at this stage than are the large majority of birds recorded in cases of pathological reduction of the ovary, and coupled with this there is a certain degree of male behavior which often is not pronounced in the pathological individuals. These observations are interesting in view of the attitude taken by Riddle² in a recent paper on "Sex-reversal in the Pigeon", where he suggests that "cases of complete sex-reversal will probably occur only in those adults which develop special diseases in particular or restricted organs."

In some of our cases individuals which have assumed more or less complete male characters as concerns head furnishings, plumage, and spurs, are reverting toward the female type as shown by the female type of plumage reappearing. This would indicate that bits of ovary may regenerate after a time, causing certain of the male characters to revert. If this be due to regenerating

⁷ Benoit, J. 1923. A propos du changement experimental de sexe par ovariectomie, chez la poule. *C. R. de la Soc. de Biol.*, 89, pp. 1326-28. Benoit, J. 1923. Transformation experimentale du sexe par ovariectomie precoce chez la poule domestique. *C. R. de l'acad. des Sc.*, 177, pp. 1074-77.

ovary then we would have to assume, that whenever sufficient ovary regenerates it would prohibit complete sex-reversal in all its characters and it may cause a reversal of some of the already formed male characters in the female direction. This statement is made with some reserve since it is not known at the present time what the internal findings will reveal. However, if ovarian tissue should be found it would seem to show that complete sex-reversal is not possible in cases of pathological reduction or natural ovariectomy unless the ovary be completely destroyed or removed.

Our results indicate that the female in the Brown Leghorn fowl has many potentialities of the male, which are normally inhibited by the presence of the ovary, and that these potentialities can assert themselves approximately fully after the complete removal of the ovary at an early age.

16 (2539)

Bacterial allergy and tissue reactions.

By HANS ZINSSER.

[*From the Department of Bacteriology and Immunity, Harvard University, Cambridge, Mass.*]

The bacterial allergies, though they are phenomena of relatively specific hypersusceptibility, differ in certain fundamental respects from true protein anaphylaxis. Our own investigations on this subject have dealt chiefly with the tuberculin reaction in regard to which it seems safe to state that:

1. Tuberculin allergy as manifested by the intracutaneous reaction may occur without general anaphylaxis to tuberculo-protein, and vice versa.
2. The active principle of tuberculin which elicits reactions in the sensitive subject is not a protein.
3. Tuberculin sensitiveness seems inseparably associated with the reactions of the animal tissues to the tubercle bacilli or their constituents; in other words, after prolonged experimentation, we arrive again at the recognition that there is no tuberculin sensitiveness without a tubercle. Here again the fact that the

tuberculin reaction is merely the classical example of the bacterial allergies in general is apparent in that our own work with other bacteria as well as especially that of Fleischner and Meyer with *bacillus abortus* has indicated that bacterial allergy is associated with infection rather than with the type of immunization with soluble bacterial substances which leads to antibody formation without necessarily active focal inflammatory reactions.

In the case of the tubercle bacillus Petroff and the writer have shown that the injection of the dead bacilli induces tuberculin allergy quite effectively, probably because of the insolubility of these organisms, in consequence of which the dead organisms lead to tubercle formation essentially analogous to that induced by the living. This point, apart from its possible practical interest, is theoretically significant in that it indicates that, in the association of bacterial allergy with infection rather than with immunization, it is the tissue reaction which is of importance rather than any differences in the products which pass into the infected animal from dead and living bacteria respectively.

Our present experiments were aimed more particularly at an inquiry into the relationship of antibodies to bacterial allergy. The problem was approached by two separate lines of investigation. In the first series of experiments pneumococcus antigen and anti-serum were employed because it was relatively easy to obtain large quantities of both substances. Considerable amounts of anti-pneumococcus serum were injected into a large series of guinea pigs by various routes and by both single and repeated injections. Subsequently, at intervals ranging from several hours to several weeks, skin reactions were done upon these animals with various forms of pneumococcus extracts, all of which were powerfully precipitable by the anti-pneumococcus serum employed for the preliminary injection. In the large majority of these animals no characteristic delayed skin reactions were obtained. In many of them mildly positive reactions appeared and in only four or five was anything approaching a tuberculin reaction observed. In other words, these experiments showed that allergy, as indicated by the delayed skin reaction, could not be produced passively in guinea pigs with any degree of certainty or regularity by the injection of highly potent specific precipitating sera, and that there was, in other words, no relationship between antibodies and allergic reactions of the tuberculin type.

In the second series of experiments tuberculin and the sera of rabbits containing precipitating antibodies for tuberculin were used. The sera were produced in various ways by the prolonged treatment of rabbits with tubercle bacilli and their products. At the same time similar passive transfers were attempted with the sera of rabbits and guinea pigs in which multiple tuberculous lesions had been produced by the injection of living tubercle bacilli, usually bovine in the cases in which rabbits were used.

The results of these experiments may be briefly summarized as follows:

1. Many of the sera containing potent precipitating properties for tuberculin and tubercle bacillus residues conveyed little or no allergy to subsequent intracutaneous administration of O. T.
2. Some of these sera did convey such allergy, which appeared four or five days after the serum injection.
3. A number of the rabbit sera obtained from rabbits which were suffering from multiple tuberculous lesions but which possessed no precipitating properties, or almost none, conveyed allergy in a typical way, so that subsequent tests with tuberculin four to eight days later resulted in + + + to + + + + (characteristic, though never necrotic) tuberculin reactions.

In a few cases in which this happened, similar allergy could be induced by the injection of the filtrates of the ground tissues of the lesions themselves.

It may be concluded that passive transfer of tuberculin allergy to normal guinea pigs can be accomplished by the injection of tissue filtrates from tuberculous lesions of rabbits and, in many cases, with the serum of such rabbits, indicating that the substance which conveys allergy is probably given off from the tissues to the serum. The presence of such substances that convey the allergy is dependent upon the existence of inflammatory foci, and varies in quantity according to factors that we have not yet ascertained. These substances, however, do not run parallel to the ordinary precipitating antibodies and can not be identified with them.

We are inclined to suggest from these experiments that allergic hypersusceptibility of the tuberculin type is dependent upon substances in the production of which tissue reactions are essential.

When dissolved or soluble bacterial materials are injected into an animal, antibodies of the classical type result which may, just as when horse serum or egg albumen are injected, lead to anaphylaxis. These antibodies in the case of bacteria are probably concerned with protection, in that by agglutination of the bacteria and the sensitization of the bacteria to phagocytic action they aid in the removal of the organism from the blood stream of the tissue spaces. No typical allergy, however, need result.

When the living bacteria are injected or, as in the case of the tubercle bacillus, dead bacteria in a more or less insoluble state, inflammatory reactions occur and, as a result of these tissue activities, other substances, not antibodies in the ordinary sense, are produced which lead to allergy.

Other work going on in this laboratory on tuberculosis indicates the correctness of the suspicion expressed by many previous workers, that tuberculin hypersensitiveness and resistance to infection are parallel processes, and these allergic substances, as we may call them for the present, therefore, may also possess a perhaps deeper protective significance.

If we remember the fact that the tuberculin reaction in its main characteristics shows close analogy with similar phenomena occurring in connection with other infections such as, for instance, the Mallein reaction, the typhoidin reaction and such work as that of Fleischner and Meyer with *bacillus abortus*, it seems more than likely that we are faced with a fundamental principle. In all of these reactions the association of the allergy with actual infection rather than with treatment with dead bacteria appears fairly clear.

Thus we cannot avoid the thought that there is a basic difference in the occurrences which result in the animal body from the injection of dissolved or soluble dead bacterial substances on the one hand, and those which are consequent upon actual inflammatory response to the living invaders. Such a conception, furthermore, is in harmony with a great many heretofore unexplained facts, such as, for instance, the difference between the results of immunization with living and with dead bacteria; the protective effects of convalescent serum in conditions in which artificially produced immune sera are relatively ineffective in spite of high antibody contents; the relatively limited protective effects of antibody containing sera in many bacterial diseases; the fre-

quent and curious lack of parallelism between antibody contents and protective functions generally. The obvious direction indicated to us for further experimentation by these results is to include attempts to produce inflammatory foci in the processes of active immunization for the production of protective sera.

17 (2540)

Estimations of blood sugar in decerebrate animals.

By H. C. BAZETT, W. Z. TYCHOWSKI, and C. CROWELL.

[*From The University of Pennsylvania Medical School, Philadelphia, Pa.*]

Figures for blood sugar following decerebration have been given by J. Mellanby,¹ but he used an injection of starch for decerebration and the actual area of brain damage remained very uncertain. He found a hyperglycemia lasting as long as six and a half hours and supposed that this high sugar level might be maintained indefinitely. On the other hand, Bazett and Penfield found a disappearance of sugar from the urine in decerebrate cats within two days.

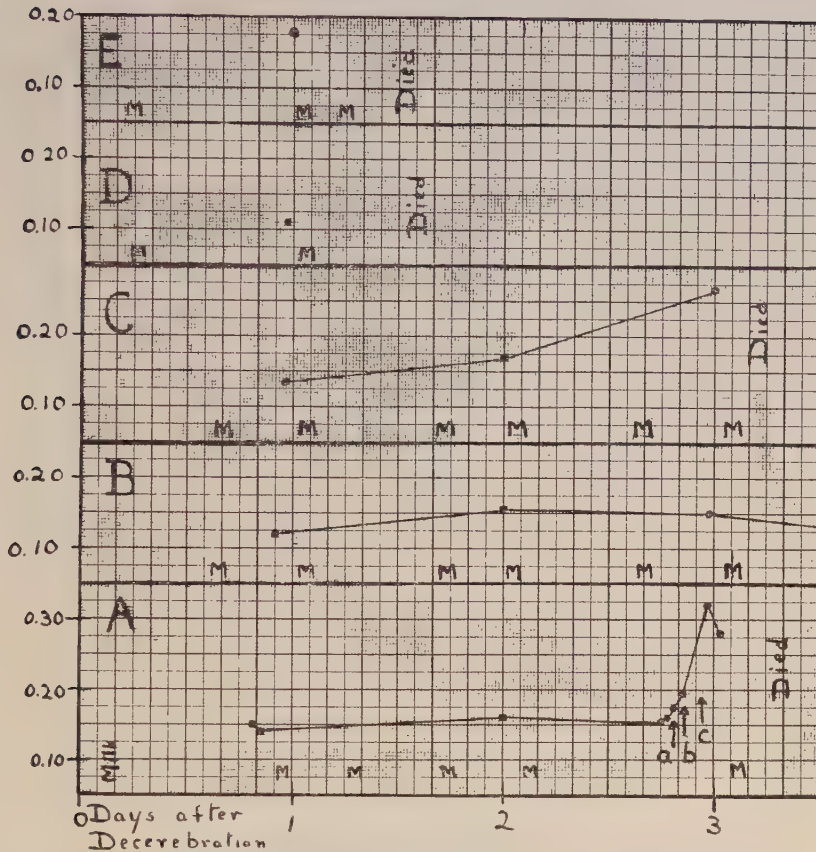
A few observations on blood sugar have been made on cats decerebrated aseptically by a scooping out method under chloroform anesthesia. The blood has been obtained from a cut in the ear and the sugar estimated by Maclean's method. In one such animal a blood sugar of 0.256 per cent was obtained 24 hours after operation, and the urine secreted during the night contained sugar. In another animal the blood sugar 24 hours after operation was found to be 0.19 per cent. In both of the above animals the pituitary was left intact. In an animal decerebrated acutely with the Sherrington guillotine and chloroform anesthesia in which the whole of the hypophysis was removed and also any accessory pituitary tissue in the pharynx a blood sugar of 0.37 per cent was obtained one half hour after decerebration and the level gradually fell until 7 hours after operation it was 0.20 per cent and finally reached 0.165 eleven and a half hours after the operation. At this time the animal began to have difficulty in

¹ Mellanby, J., *J. Physiol.*, 1919, liii, 1.

breathing from tracheal mucus, and his general condition became much worse and with this change the blood sugar rose again reaching a level of 0.38 per cent just before death, 21 hours after operation. The initial fall of blood sugar could not therefore, have been due to a lack of available glycogen. These few figures confirm the data obtained by Mellanby, but indicate that if he had carried on his experiments for a longer time the blood sugar might have fallen, and that the high blood sugar cannot be considered as maintained at a constant high level.

In further experiments animals have been decerebrated aseptically by a scoop method under anesthesia induced by chloretone given in alcohol and water by stomach tube in a dosage of 0.17 gm. per kilo, the resultant anesthesia being occasionally supplemented by a little chloroform. In these animals the hemorrhage at time of operation has been much less than with chloroform anesthesia. The chloretone has maintained its effects for 24 to 48 hours or even longer, although some rigidity has usually developed within 6 to 8 hours. In some animals the pituitary has been left intact, in others it has been removed at the time of operation and in some experiments an attempt has been made to remove pituitary, but at autopsy it has been found to have been only partially removed. The experiments have been complicated by a large number of respiratory infections probably dependent on a distemper epidemic and consequently these figures merit only a brief report. The blood sugar has been estimated after operation on 16 animals, and no difference has been noted according to the treatment of the pituitary; a normal blood sugar being usually obtained 24 hours after the operation whether the pituitary was or was not removed. The blood sugar level has not been raised in these animals by fall of body temperature, but it has been fairly constant in spite of temperature changes, and, if anything, has been lower rather than higher when the body temperature has been low.

The animals have mostly died in from 36 hours to six days, the cause of death being usually a lung or wound infection. In two cases in which the pituitary was removed the animals died in less than 48 hours with fast respiration just before death, but no obvious infection at autopsy. None of the animals in which the pituitary was left died except with very definite evidence of infection at autopsy.



DESCRIPTION OF CHART.

All decerebrated by scoop method with chloretone anesthesia. In all cases M indicates milk given by stomach tube and the time of death indicated is approximate.

A. Pituitary left intact at original operation; complete section at level of tentorium. On third day wound reopened without anesthesia and sample (a) taken after this. Then pituitary damaged with some hemorrhage after which sample (b) taken. Lastly pituitary with disconnected piece of brain lying above it removed with further hemorrhage and wound closed. Sample (c) taken 2 hours later. Death during following night probably from septicemia following second operation.

B. Attempt at pituitary removal at original operation; stalk divided and all brain above removed. Pituitary found present at autopsy. Blood sugar remained at similar level for six days. Animal died on 7th day. No definite cause of death discovered.

C. Operation same as B. Pituitary found damaged but not completely removed at autopsy. No definite cause of death found.

D. Brain removed to level of tentorium and pituitary removed. Respirations 36 twenty-four hours after operation. No definite cause of death discovered.

E. Operation and findings similar to those of D.

Our results demonstrate that the blood sugar is maintained at a normal level in cats following decerebration and that this level is not affected by removal or damage to the pituitary and that such animals are still able to raise their blood sugar when the circulation is failing just before death.

The degree and duration of the hyperglycemia appeared to be more correlated with the amount of blood clot found at autopsy below the tentorium than with any other factor. Blood sugar estimations were made also following decerebration in two animals with sodium veronal anesthesia (0.46 gm. per kilo) by stomach tube with similar results. A chart is given showing the blood sugar level in several animals following decerebration to demonstrate the stability of the blood sugar level. Experiments by one of us (W. Z. T.), included in another paper, demonstrate that the blood sugar level in normal cats is similar to that found in these decerebrate preparations and it is not normally as high as 0.2 per cent as Mellanby stated.

18 (2541)

Food accessory substances in bacterial growth. II. The variations of hydrogen ion concentration occurring in bacterial cultures containing tomato extract.

By GREGORY SHWARTZMAN, M. D. (Introduced by I. S. Kleiner).

[From the Laboratory of Bacteriology, New York Homeopathic Medical College and Flower Hospital, New York City.]

The previous observations, 5 (2528), have demonstrated that the tomato extract is not able to promote the growth of *B. Shiga* in broth of initial pH 5.2-6.2, that it has a moderate growth accelerating effect on this microorganism at initial pH 6.6-7.8 and that it has the best effect when added to broth having a pH 8.2-8.6. However, upon studying the end reaction of cultures containing tomato extract, it was observed that the final pH of these cultures, while always markedly acid, showed considerable difference, this difference depending upon the initial pH of the medium. Thus, for example, initial pH 8.2 of the tomato extract broth gives a final reading of 5.8, whereas initial pH 7.0 gives a reading of 5.2.

In this connection the work of Cohen and Clark¹ was of considerable interest. These authors found that on the acid borders of optimum zones of pH, slight change in pH produces a marked inhibitory effect upon the growth of bacteria (4.7 for *B. Shiga*), but the acid border shifts with the nature of the acid and a distinct difference between the effect of HCl and that of acetic acid was noticed. When adjustments were made with acetic acid, the narrow pH zone 5.5-5.7 may be considered *critical*, while when HCl was used, this point was 4.8-5.0.

In the author's experiments, the question, therefore, arose as to whether the organic acids contained in tomato extract shift the critical point of *B. Shiga* from 4.7 to some point quickly reached by a tomato extract culture of initial pH 6.6-7.8, but much slower, or perhaps never at all reached by a culture containing tomato extract of initial pH 8.2-8.6.

Investigation of this point demonstrated in fact that the tomato extract broth, from which the growth promoting factor has been removed by process described elsewhere,² is able to inhibit completely the growth of *B. Shiga* at pH 5.4-5.6 whereas plain broth becomes inhibitory only at pH 4.8.

As cultures containing tomato extract constantly increase their hydrogen-ion concentration, systematic study of variations occurring every hour for a period of 24 hours in these cultures, having various *initial hydrogen ion concentrations*, was necessary in order to determine the curve of pH.

Such observations showed that tomato extract cultures of initial pH 5.8 reach the critical pH 5.4 very quickly and maintain a pH level 5.4-4.8 for a period of 3 to 24 hours. Tomato extract broth cultures of initial pH 7.0 reach pH 5.4 only after 10 hours. During this period of time the pH of the culture is relatively far from the critical point. Cultures containing tomato extract of initial pH 8.2 do not reach their critical pH in the period of 20 to 24 hours.

Observations, too, on the *rate of growth* of *B. Shiga* in cultures containing tomato extract of various initial hydrogen ion concentrations showed that the decline in growth is coincident with the passing of culture beyond the critical point of pH.

¹ Barnett Cohen and William Mansfield Clark, *J. Bacteriology*, 4, 1919, p. 412.

² 19 (2542).

It is evident from the above mentioned observation that the logarithmic phase of growth of culture inoculated at initial pH 7.0 is shorter than that of initial pH 8.2. The logarithmic phase of cultures of initial pH 5.8 is again much shorter than that of initial pH 7.0, whereas it is completely abolished in cultures of initial pH 5.4. Hence the lower the initial pH, the greater the period during which the growth promoting properties of the tomato extract medium exert their influence.

To find more experimental proof for the above mentioned explanation of the influence of initial hydrogen ion concentration of media on growth promoting effect of tomato extract, a micro-organism with a narrower zone of optimum pH range than that of *B. Shiga*, such as Cholera V, was chosen.

The critical point for this organism in tomato extract medium was found to be pH 6.6-7.0. The critical point is higher than that of *B. Shiga*, hence the initial pH for the growth promoting effect of the tomato extract should be higher than that of *B. Shiga*, if the explanation given is correct. This in fact was found to be the case. It was observed that the rate of growth in tomato extract cultures of initial pH 8.2-9.0 showed considerable growth promotion, while these of initial pH 6.6-7.8 actually produced inhibition because as is seen, the cultures are in a pH where the constant increase in hydrogen ion concentration very quickly brings it to the unfavorable critical point.

19 (2542)

Food accessory substances in bacterial growth. III. Their fate in bacterial cultures.

By GREGORY SHWARTZMAN, M. D. (Introduced by I. S. Kleiner).

[*From the Laboratory of Bacteriology, New York Homeopathic Medical College and Flower Hospital, New York City.*]

Since the previous observations have shown that bacterial cultures containing tomato extract undergo a rapid change in hydrogen ion concentration leading to a considerable increase in pH, it was decided to determine whether the pH of surrounding fluid

would have anything to do with the fate of growth promoting factors in bacterial cultures.

Preliminary experiments had shown that during the first 24 hours of multiplication of *B. Shiga* in *plain broth* of various initial pH no growth promoting nor growth inhibiting substances can be detected in the surrounding fluid which could vitiate the interpretation of the following results.

Cultures of *B. Shiga* containing *tomato extract* and of initial pH 5.4, 7.0 and 8.6 and control cultures of the same pH in plain broth were centrifuged at various intervals of incubation time for a period of 24 hours, the pH of these samples recorded, adjusted to pH 8.2 and inoculated with *B. Shiga*. These experiments demonstrated that the growth promoting factors are present in fluids derived from cultures of initial pH 5.8 and 8.2 for the period of 24 hours of observation, whereas they completely disappear from tomato extract broth cultures with initial pH 7.0 as soon as the latter reaches 5.2 (10 to 14 hours). It may be pointed out that the culture of initial pH 8.2 does not reach a pH higher than 5.8 during 24 hours of incubation, while that of initial pH 5.8 naturally reaches pH 5.2 very quickly (2 to 3 hours).

What, then, are the conditions that lead to the disappearance of growth promoting factors from bacterial cultures with initial pH 7.0?

1. The phenomenon of disappearance of growth-promoting factors does not depend on *the amount* of bacteria in these cultures, since these factors persist in tomato extract cultures of initial pH 8.2 which, as was shown before,¹ contain a more abundant growth than the cultures under discussion.

2. *The length of time* during which the growth-promoting factors are in contact with living bacterial cells, does not influence the phenomenon, as they persist in cultures of initial pH 8.2 for 24 hours of observation and disappear from cultures pH 7.0 in 10 to 14 hours.

3. *An increase in the hydrogen ion concentration* of cultures containing tomato extract to pH 5.2 seems to be a necessary condition for disappearance of these factors from cultures pH 7.0.

As the pH 5.2 seems to be the only condition with which the disappearance of growth-promoting factors is associated, it

¹ 5 (2528).

seemed strange that no disappearance of these factors occurred in cultures of initial pH 5.8 which reach the pH 5.2 very quickly.

But since the initial pH 5.8, even of cultures containing tomato extract, is not favorable for the growth of *B. Shiga*, it was thought that the amount of bacterial cells is too small to bring about the disappearance of growth-promoting factors from this culture. An experiment, in which culture-medium containing tomato extract and of initial pH 5.4 was inoculated with 2 cc. of a heavy emulsion of *B. Shiga* (about 700,000,000 organisms) demonstrated that the growth promoting factors do not disappear even under these conditions.

Three possible explanations for the disappearance of growth-promoting factors from cultures of initial pH 7.0 when they reach pH 5.2, and for the persistence of these factors in cultures of initial pH 5.4-5.2 even in heavily inoculated media presented themselves, namely:

1. That although the medium pH 5.4 was seeded with a heavy inoculum, the bulk of the microorganisms may have died out,² and that it is necessary to have *living* bacterial cells at pH 5.2 in order to cause the disappearance of the growth promoting factors.

2. That the heavy inoculum at pH 5.2 does not give rise to as many *dead* bacterial cells as would be produced eventually by a culture seeded into broth of initial pH 7.0 and kept until pH 5.2 is reached.³

3. That it is necessary to have the process of *active multiplication* such as could be produced by the growth of bacteria in tomato extract culture of initial pH 7.0, and that this culture must be eventually changed to pH 5.2 in order to bring about the disappearance of growth promoting factors from bacterial cultures.

An experiment, in which a large amount of bacterial cells killed by heating to 60° for ½ hour was brought in contact with tomato extract of various pH, definitely demonstrated that the growth promoting factors cannot be removed by this process.

On the other hand a tremendous amount of *living* bacterial

² See 18 (2541).

³ In 18 (2541) it was shown that there is a pronounced death of bacterial cells when a tomato extract culture pH 7.0 reaches pH 5.2.

cells such as washings of 21 agar slants, when left in contact with $\frac{1}{2}$ cc. of tomato extract of pH 5.2 for 24 hours on ice did not bring about the disappearance of the growth promoting factors from this extract. Nevertheless, the content of living cells in this experiment must have exceeded many times that produced in tomato extract cultures of pH 7.0 receiving a small inoculum, because at initial pH 5.2 the rate of death of bacteria should leave approximately two-thirds of the original number alive in a period of 24 hours, as previous experiments had shown.

Since there remains only the third possibility it was decided to determine whether the actively growing bacterial cultures containing tomato extract and reaching pH 5.2 produce any substances which would be responsible for the phenomenon under discussion.

An experiment in which the fluid derived from a 24 hours old tomato extract culture of initial pH 7.0 and of final pH 5.2 was mixed with fresh tomato extract in proportion 1:10 and then, after being adjusted to pH 8.2, was inoculated with *B. Shiga*, demonstrated that this fluid contains certain factors which are able to destroy or inactivate a new supply of food accessory substances when brought in contact with the latter at pH 5.2. Furthermore, fluid containing inactivating substances is not able to effect the growth promoting factors if it is previously adjusted to pH 8.2 and only then brought in contact with the tomato extract.

Evidently the disappearance of food accessory substances from *B. Shiga* tomato extract cultures of initial pH 7.0 is due to the formation of unknown factors by actively multiplying bacterial cells, the actual process of inactivation being favored by pH 5.2. A study of these factors is now under way.

I am indebted to Dr. Israel S. Kleiner and to Dr. Louis Gross for constant encouragement and very valuable critical suggestions. I am thankful also to Mrs. Z. J. Gunther and Miss A. Lichtman for capable assistance.

20 (2543)

Pseudo-autotomy in albino rat.

By THEODORE KOPPÁNYI (Introduced by A. J. Carlson).

[*From the Hull Physiological Laboratory of the University of Chicago, Chicago, Ill.*]

Bayliss¹ describes the process of autotomy as follows: "If a crab be picked up by one of its ambulatory appendages, it generally, by a powerful contraction, breaks this leg off at a particular place and so obtains freedom. This mechanism was first investigated by Fredericq, and more recently by Raskam (1913). The second segment of the leg in the crab consists of two parts which are distinct members in most crustacea and united by a movable joint. In this animal, however, in place of a joint, there is a double membrane whose two components are not very firmly united. In the middle of the membrane there is an aperture, through which nerve and blood vessels pass. Certain muscles are so arranged that by a powerful contraction, they separate apart the two layers of the membrane. Thus no soft parts are torn, except the nerve and blood vessels; there is practically no bleeding and the peripheral part of the appendage is rapidly regenerated."

A similar phenomena has long been known in some vertebrates. If a lizard is held by the tail it frees itself with a quick jerk at the expense of the piece of tail which remains in the hand. This phenomenon has also been termed autotomy, but it is different from true autotomy in that the tail itself is passive and is pulled apart, whereas muscles of the crayfish leg accomplish the separation which occurs there. At Dr. Carlson's suggestion, we wish to make manifest this difference by denoting as autotomy that mechanism by which an animal suddenly discards a portion of its body by a mechanism located in the member itself, and by denoting as pseudo autotomy that process by which an animal is enabled to lose a portion of its body, the part being lost playing a merely passive rôle. There is a possibility that such a portion of the body, the lizard tail, for instance, is especially adaptive (as to be unusually friable at a certain segment) for this purpose so as to facilitate the escape of the animal.

¹ Bayliss, W. M., Principles of General Physiology, 1915.

We have observed several times that in taking hold of the tail of a pregnant albino rat or one with a litter that the animal executes a sudden gyration with its body such as described by Rabaud² in invertebrates, with the effect that one finds that the animal is free and several centimeters of tail remain in the hand. In the several animals in which this occurred, the tail fragment which was discarded amounted to about four and a half centimeters in each case. Whether this means that the conditions of holding the animal, etc., was so similar that the length of the discarded tail was the same or whether at that point the tail is weaker for such a special purpose, one can at present but conjecture.

21 (2544)

Experimental tetany and diet.

By TAKEO INOUE* (Introduced by G. R. Cowgill).

[*From the Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

In 1922 Dragstedt¹ and his co-workers were successful for the first time in preventing the onset of tetany in thyroparathyroidectomized dogs, their method being to add to the diet large amounts of lactose—from 50 to 100 grams—daily.

It has been our plan to repeat Dragstedt's work and do it more exactly and systematically, taking advantage of Cowgill's² method of feeding. In doing so we have been able to vary the food-stuff components in any way we like, keeping the total caloric value nearly the same.

After the animals were fed on a certain diet for 6 to 7 days, they were operated upon and the diet was continued. Severe tetany when it occurred usually developed on the second or the third day following the operation. When animals developed tetany, various attempts were made to cure them and special attention was paid to the efficacy of lactose in the treatment. If the animal did not show tetany under the experimental conditions, the

² Rabaud, E., *Compt. rend. de biol.*, 1923, lxxxix, 229.

* International Fellow of Rockefeller Foundation.

¹ Dragstedt, L. R., and Peacock, S. C., *Am. J. Physiol.*, 1923, lxiv, 424.

² Cowgill, G. R., *J. Biol. Chem.*, 1923, lvi, 725.

diet was changed until symptoms of tetany appeared; this was done in order to exclude any possible action of accessory glands in the experiments.

In the table are given results obtained with various diets. In each case all constituents except casein and carbohydrate were kept constant.

As will be seen in this table, in a diet relatively low in casein, the amount of lactose which may prevent the onset of tetany is comparatively small, and when casein is increased, more lactose is needed.

The dextrin experiments are significant. It is much easier to prevent the appearance of tetany or depression in thyroparathyroidectomized animals than it is to cure it, once it has become established. Although lactose administration at the time severe symptoms of tetany develop appears to give much relief after a few hours, it has never been so successful as the administration of calcium salts which cure almost all symptoms in a short time. However, by stopping the symptoms of tetany by calcium administration and then changing the diet to a lactose food mixture, we have been able usually, without giving any more calcium, to save the animals from the recurrence of tetany. Furthermore, in mild cases, we were often convinced that lactose administration alone by stomach was effective enough to cure the symptoms. During these experiments blood sera were often analyzed for calcium and phosphorus, usually once or twice a week, and in some cases several days in succession. In every case, whenever the animal showed the symptoms of tetany after thyroparathyroidectomy, the calcium content of serum was low, and a parallelism existed between the degree of lowering of the calcium and the violence of the symptoms. When the animals were kept free from manifest tetany by a lactose diet, the calcium content of blood serum at first showed a value slightly lower than normal, never below 7 mg. In the course of time after the operation this value showed a tendency to rise gradually to normal. This indicates that some compensating factor is at work to restore the calcium level to normal and may have something to do with the fact that, when the thyroparathyroidectomized dogs are kept free from tetany by suitable treatment, they gradually become less susceptible to this condition. Nevertheless the idea of the dispensability of parathyroid glands has not been appreciated so far; even the animals which were kept free from tetany more

than 40 to 60 days and which appeared to be normal, developed tetany under certain conditions, known and unknown.

On the other hand phosphorus content of blood serum in thyro-parathyroidectomized dogs was always increased regardless of whether or not the animals were kept free from tetany by lactose feeding or calcium administration. This increased phosphorus value returned gradually to normal in the course of time.

Either parenteral administration of galactose and lactose or the administration of a laxative agent (castor oil) failed to prevent the onset of tetany in thyroparathyroidectomized dogs. In experiments on normal dogs, a lactose diet raised slightly the level of calcium content in blood serum.

These experiments seem to suggest that the primary feature of tetany after thyroparathyroidectomy in dogs is a complicated disturbance of metabolism, in which protein metabolism plays some rôle. Although the lowering of blood calcium appears to be the direct cause of muscular symptoms of tetany, the calcium factor is, in our opinion, a secondary rather than a primary factor concerned in the development of the characteristic syndrome of experimental tetany. Just how lactose acts in preventing tetany still remains for further investigation. Possible changes in the permeability of the alimentary tract in this connection may require additional attention.

Diet and Parathyroid Tetany.

Tetany			Boundary			No Tetany		
No. Dogs	Diet.		No. Dogs	Diet		No. Dogs	Diet	
5	Standard	Per cent	3	Lactose <i>d</i>	Per cent	3	Lactose <i>a</i>	Per cent
	Casein	37.6		Casein	5.5		Casein	5.5
	Sucrose	34.9		Lactose	5.0		Lactose	68.1
4	High glucose			Sucrose	63.1	2	Lactose <i>b</i>	
	Casein	5.5	2	Casein-lactose <i>d</i>			Casein	5.5
	Glucose	68.1		Casein	20.0		Lactose	26.6
1	Sucro-glucose			Lactose	5.0		Sucrose	41.5
	Casein	5.5		Sucrose	53.1	4	Lactose <i>c</i>	
	Glucose	27.0	3	Casein-lactose <i>b</i>			Casein	5.5
	Sucrose	41.1		Casein	40.0		Lactose	15.0
3	High sucrose			Lactose	15.0		Sucrose	53.1
	Casein	5.5		Sucrose	23.1	3	Casein-lactose <i>a</i>	
	Sucrose	68.1					Casein	37.6
3	High dextrin						Lactose	34.9
	Casein	5.5						
	Dextrin	68.1						
3	Casein-lactose <i>c</i>							
	Casein	40.0						
	Lactose	5.0						
	Sucrose	33.1						

22 (2545)

An experimental study of the origin of the meninges.

By SAMUEL C. HARVEY, M. D., and HAROLD S. BURR, Ph.D.

[From the Departments of Surgery and Anatomy, Yale University,
New Haven, Conn.]

As a result of experimental work concerning the healing of the meninges following injury, it was found by one of us (S. C. H),¹ that the dura in the presence of an intact lepto meninx heals without adhesion to the underlying membrane. This is brought about, not by the ingrowth of mesothelium from the surrounding normal dura, but by the direct transformation of mesenchymal cells into mesothelium which comes to lie against the uninjured and impervious lepto-meninx.

On the other hand,² when the lepto-meninx is injured with the overlying dura remaining undamaged, an analogous healing without adhesions does not take place. The lining mesothelium of the dura disappears and this membrane enters intimately into the reparative process in the subjacent lepto-meninx, the final result being dense adhesions. This seems to indicate some fundamental histogenetic difference between these two structures.

Harrison³ has shown by experimental methods that the nerve sheath cells are derived from the neural crest. Inasmuch as these cells are analogous to the lepto-meninx in their relationship to the nerve structures which they surround, it seemed possible that this membrane was likewise derived from the neural crest while the dura probably developed from the mesenchyme.

An examination of pig and chick embryos at and previous to the time of the outgrowth of the nerve root fibers from the neural tube, confirmed this belief.

It seemed possible to verify this supposition by experimentation. This work was carried out by the transplantation of sections of the neural tube of the amblystoma with and without neural crest cells. If the lepto-meninx develops from neural crest cells, then transplanting a portion of the neural tube free

¹ Sayad, William Y., and Harvey, Samuel, *Annals of Surgery*, 1923, xxxvii, 129.

² Lear, Maxwell and Harvey, Samuel, *Annals of Surgery*, in press.

³ Harrison, Ross G., *Am. J. Anat.*, 1906, v, 121.

from such cells should lead to the growth of neural tissues surrounded only by dura, while such transplantation carrying with it nerve crest cells should lead to the formation of both the dura and the lepto-meninge about the neural transplant. Such experiments were carried out in the Spring of 1924, with the following results.

The first series was that in which the mid-brain, optic vesicle and the adjacent neural crest cells were transplanted into the region lying just anterior to the limb. The result of the procedure was that the transplanted neural tissue was completely invested by lepto-meninge and dura.

The second series involved the transplantation of a portion of the cerebral hemisphere into the same region, after careful removal of neural crest cells. Examination of the result showed the growth of neural tissue with no lepto-meninge, it being surrounded only by an incompletely formed dura.

These experiments indicate then that there is a definite histogenetic difference between the dura and the lepto-meninge, and that the latter has the same origin as that of the neural sheath cells, namely, the cells of the neural crest, while the former is probably developed from mesenchyme.

ABSTRACTS OF COMMUNICATIONS.

Pacific Coast Branch.

Special Meeting with the A. A. A. S.

Stanford University, June 25, 1924.

23 (2546)

Regulation of the hydrogen ion concentration and its relation to metabolism and respiration in the starfish.

By LAURENCE IRVING (Introduced by E. G. Martin)

[From the Laboratory of Physiology and the Hopkins Marine Station of Stanford University, San Francisco, Calif.]

In the starfish *Pisaster ochraceus* and *Patiria miniata* the coelomic fluid was found to have a pH about 7.6, compared with the normal for sea water of 8.3. The pyloric caeca, which are naturally immersed in coelomic fluid, were found to have a pH of 6.7. All determinations were made colorimetrically and are given without correction.

Excised caeca, when suspended in a small amount of sea water, gradually altered the pH toward 6.7. Even when the sea water was changed from its natural pH by acidification and subsequent aeration till equilibrium was established, the caeca altered the pH to 6.7 when the water was initially at any pH between 8.6 and 6.3. An excellent criterion of survival of the caeca is presented by the persistence of ciliary activity of the epithelium. These cilia maintain their activity for about 48 hours on excised caeca where the initial pH of the medium is between 8.6 and 6.3. The optimum pH for survival is 6.7, the same as found normal for the caeca. If the pH was initially less than 6.3, the cilia soon ceased and the caecum disintegrated, and any caeca which fell below 6.7 were dying and approached 6.3 as the apparent natural point for disintegration.

Determinations of the rate of digestion of gelatine solutions introduced into excised caeca showed an optimum at about 6.7. The degree of digestion was followed by Van Slyke's method for amino-nitrogen, taking samples from the medium surrounding the caeca after the gelatine had been introduced. In the controls it appeared that scarcely any amino-nitrogen is produced naturally by the caeca in spite of the intense ciliary activity. As CO_2 is produced quite abundantly, oxidation of fat is suggested as the source of energy.

Using the pH change as a proper measure of CO_2 , it was apparent that CO_2 production was most vigorous at pH about 6.7. In the more alkaline media CO_2 production is gradually diminished with further departure from this normal pH. Below pH 6.7 the production appears practically to cease, a condition coincident with rapid death.

The existence of an optimum condition of hydrogen ion concentration at a point different from sea water shows the existence in the organism of a regulatory process. In the production of this optimum either added HCl or the H_2CO_3 produced by its own metabolism is effective in securing the favorable condition. It appears from the fact that some of the favorable media were in equilibrium with the CO_2 of the air, while others contained a higher tension of CO_2 , that the optimum point is not a matter of increased facility of CO_2 elimination because of the development of a greater CO_2 tension within the caeca. It is apparently a true optimum for CO_2 production by the tissue itself, irrespective of the particular conditions of CO_2 elimination.

24 (2547)

The carbonic acid-carbonate equilibrium in sea water.

By LAURENCE IRVING (Introduced by E. G. Martin).

[*From the Laboratory of Physiology and the Hopkins Marine Station of Stanford University, San Francisco, Calif.*]

Ca, Mg, and CO_2 in sea water are the common constituents which are most variable in quantity. The condition of these substances is sensitive to the slight changes of the ocean, in con-

trast to the stability of soluble neutral salt forming substances. They are, furthermore, concerned in a direct way with vital reactions. CO_2 is also one of the most important indicators of vital activity and the suitability of the solution for organisms because it is a product of destructive metabolism and a means for the fixation of energy. If the carbonic acid-carbonate system is considered distinctly, it is conspicuous first that it requires time to equilibrate sea water with a gas phase containing CO_2 . If acid or alkali is added to sea water, a number of hours aeration will be required before a constant pH is attained with any tension of CO_2 .

Bubbling and shaking sea water with pure hydrogen slowly increases the pH to about 9.2. This is about the point found by Atkins and others as the limit for photosynthetic activity of several marine algae. After acidification and bubbling to remove CO_2 , an alkali titration curve lags below the curve for a base of the same concentration as the excess base of sea water (0.0025 N). Such a curve shows the buffer effect of non-volatile buffers alone. A direct acid titration shows the combined buffer effect of both volatile and non-volatile weak acids.

Titration curves at various CO_2 tensions differ from those for pure carbonate solutions in requiring more acid. Taking this difference as a measure of the buffer effect of other weak acids than carbonic, their apparent dissociation constant is calculated as $k = n \times 10^{-8}$. L. J. Henderson's artificial sea water was 0.0015 M in boric acid, but boric acid is not reported so abundant in sea water. Silica is reported in a concentration sufficiently large to have this effect if it behaves like an acid as weak as boric. This point is not revealed with the existing data, but there is suggested an important topic because of the presence of silicon in many organisms and geological processes.

Because sea water contains other weak acids than the volatile carbonic acid, its pH may be varied by the addition of acid and the change fixed at any CO_2 tension if aeration with CO_2 at that tension is continued until the equilibrium is established. If the equilibrium condition is not considered, there can be no determination of the effect of various water samples used on metabolism because of the uncertain resistance of the solution to CO_2 elimination.

25 (2548)

The formation of lactic acid by depancreatized dogs.

By EDWARD A. DOISY, A. P. BRIGGS, C. J. WEBER and
IRENE KOECHIG.

[*From the Departments of Biological Chemistry and Medicine of
St. Louis and of Washington University Schools of
Medicine, St. Louis, Mo.*]

In seeking to extend our observations,¹ that the concentration of lactic acid is increased in the blood of normal dogs following the injection of insulin, we have studied the blood of depancreatized dogs during the violent exercise of strychnine convulsions. In an earlier investigation dealing with this problem Sass² came to the conclusion that, in contrast to normal animals, the titratable alkalinity of the blood of depancreatized dogs is decreased much less by strychnine convulsions, and that this is probably due to an impaired capacity to form acid (lactic) from carbohydrate.

Barr^{3, 4} and his collaborators have recently published investigations showing that patients with a partial diabetes and completely phlorhizinized dogs form lactic acid as a result of exercise. Our experiments supply data on the same point for complete pancreatic diabetes in dogs and give additional information on the quantity of lactic acid precursor and glycogen in the muscles.

Experimental procedure: Male dogs were depancreatized and kept on insulin until the wound had healed. About ten days after the operation, the insulin was withdrawn. Within 4 to 6 days the urine generally showed a D:N ratio of about 2.8. A day or two later strychnine convulsions were induced and blood taken for comparison with a control sample. Since the blood specimens were generally taken from the femoral artery the figures given in the table are the concentrations in the arterial blood.

¹ Briggs, A. P., Koechig, I., Doisy, E. A., and Weber, C. J., *J. Biol. Chem.*, 1924, lviii, 721.

² Sass, M., *Zt. f. exp. Path. u. Ther.*, 1914, xv, 370.

³ Himwich, H. E., Loebel, R. O., and Barr, D. P., *J. Biol. Chem.*, 1924, lix, 265.

⁴ Loebel, R. O., Barr, D. P., Tolstoi, E., and Himwich, H. E., *J. Biol. Chem.*, 1924, lxi, 9.

TABLE I.
Formation of Lactic Acid by Depancreatized Dogs.

Before strychnine			After strychnine				Remarks.
Dog No.	Glucose	Lactic Acid	First sample		Second sample		
			Glucose	Lactic Acid	Glucose	Lactic Acid	
1	170	26.	173	87.5	285	178.	No microscopic examination made.
2	337	31.	361	51.	364	42.	D/N 2.7 on day of experiment. No generalized convulsions.
	428	29.5	430	63.	418	89.5	D/N Apr. 13, 2.75; 14th, 2.72; 15th, 2.83; strychnine on Apr. 16th and again on Apr. 18th. Two small nodules of pancreas found on microscopic examination.
	403	37.5	389	71.	389	97.5	
3	345	35.5	345	81.	350	113.	D/N Apr. 13, 2.75; 14th, 2.86; 29th, 2.82; strychnine on Apr. 28th. Minute quantity of pancreas in duodenal wall.
	308	41.7	326	59.8	337	82.9	D/N for 4 days preceding strychnine convulsions varied between 2.76 and 2.30. No pancreatic tissue on microscopic examination. Post-mortem lactic acid in muscle 0.422 per cent.
4	263	42.4	267	62.8	270	82.7	
	500	41.6	500	63.0	550	94.4	D/N for 4 days preceding first strychnine convulsions varied between 2.86 and 2.23. No pancreatic tissue on microscopic examination. Post mortem lactic acid in muscle 0.437 per cent.
5	421	39.5	421	47.9	430	113.4	
6	359	27.8	326	66.8			Post mortem lactic acid in muscle 0.172 per cent.
							Lactic acid maximum of muscle in NaHCO_3 0.125 per cent. Second blood sample taken from heart 1 minute after death.
8	265	16.0	292	39.	235	89.	
							Lactic acid maximum of muscle 0.150 per cent. Contractions in response to strychnine poor.
9	192	34.0	181	36.	176	36.	

After the death of each dog an examination of the abdomen for pancreas was made. All suspicious tissue fragments and the upper end of the duodenum were sectioned, stained and examined microscopically by the Pathology Department of St. Louis University. The experimental results reported in this paper were obtained on dogs that had only minute residues of pancreas or none at all. There seems to be no doubt about their ability to form lactic acid in response to strychnine convulsions.

In Table I we have given the results of 12 experiments performed on eight depancreatized dogs. Eleven of the 12 experiments show that lactic acid is formed during the convulsions induced by strychnine. Dog 9 differs from the others in that the blood sugar values were much lower, and that no increase in the lactic acid was observed. This dog had received several injections of adrenalin and was very weak. We believe that the convulsions were not severe enough to pile up the lactic acid in the arterial blood.

We are inclined to the belief that depancreatized or phlorhizinized dogs form lactic acid during muscular work so long as their muscles possess glycogen. As the store of glycogen is depleted, the animal becomes less able to produce lactic acid and perform work. (Compare experiments on Dogs 8 and 9 with 4 and 5).

Although the values of the lactic acid maximum of Dogs 8 and 9 are only about one-third of the value for a well nourished dog, they indicate the presence of glycogen. Direct determinations showed that some glycogen was still present even after the severe treatment given these dogs.

CONCLUSIONS.

Depancreatized dogs form easily detectable quantities of lactic acid during strychnine convulsions. Even though these animals may be unable to oxidize carbohydrate, still the production of lactic acid seems intimately concerned with muscular contractions.

26 (2549)

The effect of grinding upon starch and starch pastes.

By C. L. ALSBERG and E. E. PERRY.

[From the Food Research Institute, Stanford University,
San Francisco, Calif.]

Several investigators have studied the effect of grinding upon raw starch. Schleiden¹ reported that grinding starch with twice its weight of water gave the mass a salve-like consistency. Kraemer² found that grinding with sand and Sponsler³ that grinding dry in a pebble mill rendered part of the starch soluble in cold water. These observations were verified by the writers. Grinding potato starch for 74 hours with flints in a pebble mill and wheat starch for 122 hours injures all the grains, as may be noted with the microscope. About 60 per cent of each of the starches was then soluble in cold water. The solution could be filtered quite clear with paper pulp, was not viscous, and gave a characteristic blue iodine reaction. Apparently the starch grain is protected by a membrane or membrane network impermeable to the soluble substance. When the membrane structure is injured, the contents may be leached out with the greatest ease. Ground starches suspended in water and heated do not gelatinize, and no paste can be obtained. The viscosity of suspensions of ground starch determined between 20° and 95° by means of a Stormer viscosimeter was relatively little more than that of water.

Stiff starch pastes made by boiling 5 per cent of untreated wheat starch in water were ground cold in a pebble mill from 13 to 16 hours and their viscosity tested before and after grinding over a range of temperature from 50° to 90°. After grinding the viscosity was reduced to about one-fourth of its initial value.

¹ Schleiden, J. M., *Principles of Scientific Botany*, p. 13. Translated by E. Lankester. London, 1849.

² Kraemer, H., Further observations on the structure of the starch grain. *Botanical Gazette*, 1905, xl, 305.

³ Sponsler, O. L., The structure of the starch grain, *Am. J. of Botany*, 1922, ix, 471.

Beijerinck⁴ ground boiled potato starch with sand, thus rupturing the membranes. He found that this treatment rendered the starch in part soluble, 60 per cent passing into solution. This agrees very well with the observation above reported in regard to the solubility in cold water of ground unheated starch. The solubility of raw starch in cold water when ground indicates that the observation of Beijerinck does not depend upon depolymerization by heat and water but that soluble starch as such is present in considerable amount in the natural untreated starch grain.

The observations herein recorded offer, it is believed, a key to an explanation of some of the physical properties of gelatinized starch and starch paste as well as of some of the phenomena observed in the gelatinization of starch granules. They must be taken into consideration in future studies on the structure and composition of the starch grain. These and other implications of the observations are still under investigation.

27 (2550)

Further evidence of the role of hepatic internal secretions in canine anaphylaxis.

By W. H. MANWARING, J. R. ENRIGHT, DOROTHY F. PORTER
and H. BING MOY.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, San Francisco, Calif.*]

All parts of the gastro-intestinal tract except the oesophagus are thrown into contraction, during typical anaphylactic shock in dogs. These contractions usually begin after a latent period of about forty-five seconds. They usually reach their maximum by the end of two and a half minutes.

The contractions vary in intensity in different parts of the gastro-intestinal tract. In the stomach, they usually increase the

⁴ Beijerinck, M. W., Structure of the starch grain. *Koninklijke Akademie van Wetenschappen te Amsterdam*. Proceedings of the Section of Sciences, 1912, xiv, 1107.

intra-gastric pressure about 10 mm. Hg. In the small intestines, a pressure increase of about 25 mm. Hg. is usually observed. In the colon and rectum, an increase of at least 50 mm. Hg.

Gastro-intestinal contractions similar to these are produced by the intravenous injection of histamine.

These contractions of the gastro-intestinal tract do not take place on travenous injection of specific foreign protein into dehepatized sensitized dogs. In contrast with this finding, dehepatization does not abolish the typical gastro-intestinal contractions to histamine.

The gastro-intestinal contractions in intact anaphylactic dogs are not secondary to the anaphylactically decreased arterial blood pressure. Rapid exsanguination, reproducing the characteristic blood pressure fall of canine anaphylaxis, does not throw the gastro-intestinal tract into contraction, at least during the period of our tests (five minutes). The gastro-intestinal contractions in intact anaphylactic dogs are also not secondary to local passive congestion. Ligation of the portal vein, producing a more extreme passive congestion of the gastro-intestinal tract than that observed during typical anaphylactic shock, does not increase the gastro-intestinal tone during the period of our tests. We, therefore, believe the gastro-intestinal contractions in intact anaphylactic dogs, similar to the urinary bladder contractions previously reported,¹ are caused by chemical products (hepatic anaphylatoxins), explosively formed or liberated by the anaphylactic liver, products having a histamine-like effect on the gastro-intestinal musculature.

¹ Manwaring, W. H., Hosepian, V. M., Enright, J. R., and Porter, Dorothy F., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 284.

28 (2551)

The fundamental physiological mechanism of anaphylaxis.

By W. H. MANWARING.

[From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, San Francisco, Calif.]

The following is our conception of the fundamental physiological mechanism of canine anaphylaxis, and constitutes our present working hypothesis with other animal species.

We assume that the capillary endothelium is the initial point of anaphylactic attack and defense. We further assume that in dogs the different capillary systems have different susceptibilities to the anaphylactic insult, the capillaries of the liver having the greatest susceptibility.

On intravenous injection of the usual doses of specific foreign protein into sensitized dogs, the hepatic endothelium alone is unable to resist the anaphylactic insult. There is a sudden increase in capillary permeability in this organ, with the rapid passage of foreign protein and altered blood plasma into the hepatic tissue spaces.

The hepatic parenchyma, in consequence, is immediately thrown into exaggerated, possibly atypical functional activity. The chemical products from this activity pass rapidly into the circulating blood, on account of the increased hepatic capillary permeability. These products, acting in conjunction with the circulating foreign protein and altered blood plasma, break down the endothelial defenses in the extra-hepatic tissues, with the resulting rapid passage of atypical products into the extra-hepatic tissue spaces.

This conception, we believe, fully accounts for the clinical picture of canine anaphylaxis. Thus, the suddenly increased local tissue pressure from hepatic edema with the resulting passive narrowing of the hepatic capillaries, coupled with the suddenly increased local blood viscosity from plasma loss, would account for the characteristic hepatic stasis. The suddenly reduced available blood volume from hepatic stasis and resulting gastro-intestinal passive congestion, coupled with the reduced blood volume from hepatic and extra-hepatic plasma losses, would account for the characteristic fall in arterial blood pressure.

This conception of the fundamental mechanism of anaphylaxis tends to unify anaphylactic phenomena in different animal species. The dominant clinical manifestations in different species would depend upon the relative anaphylactic susceptibility of the capillary endothelium in these species, with the resulting promptness and completeness with which atypical chemical products are brought into contact with the secondary tissues.

29 (2552)

An experimental study of the effect of stenosis upon the respiratory changes induced by muscular exercise.

By A. W. HEWLETT and (by invitation) J. K. LEWIS and
ANNA FRANKLIN.

[From Stanford Medical School, San Francisco, Calif.]

Dyspnea from muscular exercise results when the increased respiratory demands can no longer be met without effort. In the present series of experiments the ability to meet these demands was artificially restricted by having a normal individual (A. W. H.) breathe through small apertures. For this purpose two perforated corks were used, the diameters of the bores being 8 mm. and 6 mm. respectively. The first of these caused no effort so long as the subject of the experiments was at rest. It caused moderate respiratory effort when stairs were climbed at the rate of approximately 80 per minute. The second caused slight effort at rest and considerable distress during stair climbing. The latter culminated in discontinuance of the exercise after two or three minutes.

During rest, the respiratory rate was not influenced by the milder obstruction but in some experiments was somewhat slowed by the more marked obstruction. During exercise, the respiratory rate was definitely slowed by each, average figures being: without stenosis 27 respirations per minute, with 8 mm. stenosis 21 respirations per minute, and with 6 mm. stenosis 17 respirations per minute.

During rest, the minute volume of respiration was not altered by the lesser stenosis but in some experiments became somewhat

less with the more marked stenosis. During exercise, the minute volume was regularly reduced. After one to three minutes of exercise pulmonary ventilation became approximately constant at the following levels: without stenosis 52 liters per minute, with 8 mm. stenosis 46 liters per minute, and with 6 mm. stenosis 28 liters per minute. With the more marked obstruction, stair climbing had to be discontinued after 2 to 3 minutes on account of increasing distress. With the less marked obstruction this was not the case. Compensation for the restricted ventilation was here accomplished by increasing the percentage of O_2 absorbed from and of CO_2 given off to the respired air.

Comparative observations of the respiratory activities were made during and shortly after the ascent of 100 steps. Pulmonary ventilation during the one and a quarter minutes of this exercise became less as the obstruction increased; yet the ventilation immediately after the exercise showed no significant alteration as a result of the obstruction. The CO_2 output during the exercise was progressively diminished by the obstructions, the excess being eliminated during the first few minutes of recovery. The oxygen absorption was not appreciably influenced by the lesser obstruction but it was reduced during exercise by the more marked obstruction, the relative deficit being made up during the first two minutes of recovery.

In these experiments degrees of stenosis were used which produced little or not effect during rest but caused both subjective and objective disturbances of respiration during exercise. The most constant objective effects of stenosis were the relatively slower respiratory rate and the relatively smaller pulmonary ventilation when exercise was taken. During a short period of exercise there was a lessened output of CO_2 and in the case of the more severe obstruction a lessened absorption of oxygen, which changes were equalized during the first few minutes of recovery. Where prolonged exercise could be tolerated, compensation for the reduced volume of ventilation was effected by increasing the percentages of O_2 absorbed and of CO_2 eliminated. The pulmonary ventilation during recovery from short periods of exercise gave no indication of the degree of stenosis or of the subjective dyspnea.

ABSTRACTS OF COMMUNICATIONS.

*Western New York Branch.***Thirteenth Meeting.***Rochester, New York, October 11, 1924.***30 (2553)****The influence of insulin on the respiratory metabolism of normal rabbits.**

By ESTELLE E. HAWLEY (Introduced by John R. Murlin).

[From the Department of Vital Economics, University of Rochester, Rochester, N. Y.]

The data to date on the respiratory changes resulting from insulin injections are conflicting. It is generally agreed that insulin causes a rise in R. Q. when administered to normal subjects but just how this change is brought about is still a matter of discussion. Is the increase in quotient due to increase in CO_2 , decrease in O_2 , or both?

Normal healthy rabbits were used as the experimental animals in this series of experiments. All were carried out under the same conditions as to care, diet, and time elapsing between food and experiments. Great effort was made to obtain comparable results. The blood sugar curve coincident with the respiratory change was followed. This also served as a check on the potency of the insulin.

From the findings to date the following summary seems justified:

1. Insulin, when administered to normal animals brings about, first a slight decrease, then a decided increase in the R. Q. curve, reaching the peak two hours after injection and returning to the basal level four hours after.
2. A blood sugar drop accompanies the R. Q. change, the lowest blood sugar occurring at the peak of the curve. The return to normal is less rapid than the return to normal of the R. Q. though the curve is well on its return by the end of the four hours.

3. There is, in the second hour, both a decrease in the O_2 and an increase in the CO_2 , both changes tending to increase the R. Q.

4. The O_2 consumption and the heat production would indicate that the total metabolism is not markedly increased.

31 (2554)

The influence of insulin in phloridzin diabetes.

By O. H. GAEBLER and JOHN R. MURLIN.

[*From the Department of Vital Economics, University of Rochester, Rochester, N. Y.*]

In the hope of finding a more favorable subject than the depancreatized animal for study of the influence of insulin administered orally, twelve dogs were used after the establishment of the typical D:N ratio of Lusk. Insulin produced in the laboratory by the amyl alcohol method,¹ administered subcutaneously, caused not only a sharp decline in the excretion of sugar (and nitrogen), but also a rise in respiratory quotient from diabetic level (0.69) to as high as 0.85. Sugar was administered by stomach both in control and in insulin periods. In several instances after a dose of 1 R. U. per two kilos dog the R. Q. rose within the first two hours only to 0.77 or 0.78 and immediately dropped back to diabetic level. The effect on the excretion of nitrogen was sometimes parallel with and in a few instances greater than the effect on the excretion of sugar.

Insulin was given orally in several different combinations: in cod-liver oil emulsion (no effect); in oleic acid suspension (no effect); in blood serum (some effect). The best results (rise in R. Q. to 0.77 or 0.79) were obtained after the use of 1 R. U. per kilo in the form of an enteric coated tablet containing insulin and malic acid.² The effect on the excretion of sugar was not so marked even with this tablet as after subcutaneous administration.

¹ Allen, Piper, Kimball and Murlin, *PROC. SOC. EXP. BIOL. AND MED.*, 1923, xx, 519.

² Murlin, Sutter, Allen and Piper, *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 338.

There is evidence of retention of sugar as well as of combustion in some of the experiments both after subcutaneous and after oral administration. Whether the effect on combustion is direct or indirect these experiments do not permit us to say.

32 (2555)

The influence of insulin administered by alimentary tract on the blood sugar of etherized and adrenalectomized animals.

By H. A. PIPER and JOHN R. MURLIN.

[From the Department of Vital Economics, University of Rochester, Rochester, N. Y.]

Numerous experiments on some twenty-five dogs and half a dozen cats have been performed in the attempt to demonstrate clearly the absorption of insulin from the alimentary tract. This is not difficult when the insulin is placed directly in the intestine. A sharp drop in the hyperglycemia of ether anesthesia is easily obtained. But when insulin is given by mouth to animals treated with ether or adrenalin or both the demonstration often fails because when it is adequately protected against the stomach, absorption of the insulin from the intestine is much retarded and controls are difficult to establish. An interval of at least five days is necessary in order to insure a return of the glycogen storage to normal, and after half a dozen treatments with ether for several hours at a time there seems to be permanent impairment of the capacity to store glycogen. Alcohol up to 20 per cent and in amount sufficient to thoroughly intoxicate does not materially improve the absorption.

Some advantage clearly was gained from the administration of insulin both in solution and in enteric coated tablets, combined with sodium oleate.

33 (2556)

Observations on an ascospore stage for the parasites of blastomycosis hominis.

By RALPH R. MELLON, M. D.

[From the Department of Laboratories, Highland Hospital, Rochester, N. Y.]

An ascospore stage in the life history of the organism of blastomycosis has not hitherto been recognized. In fact as great a mycological authority as Vuillemin¹ has included these organisms in the provisional genus *Cryptococcus* in which asci have never been observed.

These distinctive forms occurred exclusively in the so-called secondary colonies of the cultures, which also contained "dauerzellen" and pigmented oidia. The prolonged viability of the cultures is associated with these secondary colonies, while the primary non-pigmented growth derived from them is seldom viable after a month.

Certain forms pictured in the tissues by Wade² are quite comparable with these forms of special resistance in the cultures and form a plausible explanation of the great difficulty of eradication of the disease by therapeutic measures. Inasmuch as oidial stages are common to so many fungi, it may be in order to suggest allocation of the so-called *oidiomyces* (*blastomyces hominis*) with the ascomycetes.

¹ (Vuillemin) Besson, A., Practical bacteriology, microbiology and serum therapy. N. Y. Longmans, 1913, pp. 706-707.

² Wade, H. Windsor. Variation of gemmation of *blastomyces dermatidis* in the tissue lesion. *J. Inf. Dis.*, v. 18, pp. 618-629, 1916.

34 (2557)

An experiment on the effect of sodium bicarbonate and of intarvin on the excretion of acetone.

By ROGER S. HUBBARD and FLOYD R. WRIGHT.

[*From the Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.*]

Haldane¹ has shown that if large doses of sodium bicarbonate (0.6 gm. per kg. body weight) are fed to normal subjects receiving normal diets the metabolism of glucose is depressed and acetone bodies appear in the urine. An experiment was planned to determine whether an increased excretion of acetone could be induced by moderate doses of the alkali in an arthritic subject who was receiving a diet which furnished sufficient calories for her needs (the patient gained in weight during the period of study) but which contained such small amounts of carbohydrate as just to prevent any significant ketosis. A markedly increased excretion of acetone was induced by 1.3 gms. (20 grains) of sodium bicarbonate fed in three equal doses after each meal. The increased excretion lasted as long as the drug was continued (August 11 to 18). The diet was approximately constant throughout the experiment except on the 17th and 18th of August, when the carbohydrate was somewhat decreased through an error. After this experiment part of the fat in the diet was replaced by a synthetic fat containing fatty acids with an odd number of carbon atoms (intarvin). A reduction in the amount of acetone excreted was found on the 22nd and the 23rd, as would be expected from the work of Kahn.² The carbohydrate content of the diet was then slightly decreased, and again intarvin was substituted for part of the fat (August 28) and an even greater decrease in the excretion of acetone than before was found. In this experiment on a subject maintained at the borderline of ketosis therapeutic doses of sodium bicarbonate caused an increased, and the synthetic fat intarvin a decreased excretion of acetone.

¹ Haldane, J. B. S., Influence of hydrogen ion concentration-changes on human metabolism. Abstracts of Communications to the XIth International Physiological Congress held at Edinburgh July 23-27, 1923.

² Kahn, M., Odd carbon atom fats in the treatment of diabetic ketosis. *Am. J. Med Sci.*, 1923, clxvi, 826.

Date	Protein	Fat	Carbohy- drate	Per cent of calories as carbohy- drate	Sodium bi- carbonate	Volume cc.	Acetone bodies as acetone		
							Preformed plus aceto- acetic acid mg./100 cc.	B-hydroxy butyric acid mg./100 cc.	Total gm.
1924.									
Aug. 6	54	170	54	12	0.0	2720	0.0	0.0	0.000
" 7	54	170	55	12	0.0	1080	0.7	1.0	0.018
" 8	54	169	55	12	0.0	2520	0.7	0.7	0.036
" 9	56	170	53	12	0.0	1820	0.5	0.0	0.009
" 10	55	169	54	12	0.0	2320	0.7	0.4	0.017
" 11	56	168	54	12	0.0	1540	6.0	6.9	0.198
" 12	54	170	54	12	1.3	3140	3.2	7.4	0.332
" 13	55	169	56	12	1.3	2540	4.2	3.7	0.201
" 14	55	171	54	12	1.3	2310	8.0	14.5	0.520
" 15	56	171	54	12	1.3	2060	14.6	18.0	0.667
" 16	57	170	54	12	1.3	1960	6.8	11.6	0.361
" 17	54	175	46	10	1.3	1820	10.3	11.9	0.405
" 18	54	175	44	10	1.3	2400	6.0	4.7	0.257
" 19	55	170	54	12	0.0	2500	5.7	5.3	0.275
" 20	55	172	55	12	0.0	1720	3.4	2.2	0.096
" 21	53	171	55	12	0.0	2070	2.8	3.1	0.122
" 22	54	168*	55	12	0.0	2110	1.6	1.2	0.059
" 23	55	170*	56	12	0.0	1690	2.1	1.1	0.054
" 24	54	170	54	12	0.0	1160	8.5	12.4	0.243
" 25	56	171	56	12	0.0	1790	3.5	2.2	0.102
" 26	54	175	46	10	0.0	2580	3.0	2.8	0.149
" 27	54	175	45	10	0.0	1980	4.0	5.3	0.184
" 28	55	175*	46	10	0.0	2400	0.7	0.4	0.027
" 29	55	174	44	10	0.0	1980	2.7	2.4	0.103
" 30	54	175	46	10	0.0	1340	3.7	5.7	0.107
" 31	55	169	55	12	0.0	1870	2.4	1.8	0.059

* On these days 60 grams of intarvin was substituted for an equal amount of fat.

35 (2558)

Comparative study of the sugar concentration in arterial and venous blood during insulin action.

By CARL F. CORI and GERTY T. CORI.

[From the State Institute for the Study of Malignant Disease,
Buffalo, N. Y.]

It has been shown by Cori, Cori and Goltz¹ and by Frank, Nothman and Wagner² from the Minkowski Clinic that by comparing the sugar concentration in the femoral artery and femoral vein of rabbits starved for 24 hours, an increased disappearance of sugar from the blood into the muscles can be demonstrated during insulin action. In view of these results it seemed desirable to investigate whether animals receiving glucose plus insulin would not show a stronger intake of sugar by the muscles than animals which received the same amount of glucose but no insulin. As a standard dose 5 gm. glucose per kgm. was given by stomach tube to rabbits previously starved for 48 hours. The insulin was injected intravenously in a dose sufficiently large to prevent a rise in bloodsugar after the glucose ingestion. Blood samples were taken simultaneously from the femoral artery and femoral vein of unanesthetized animals in half hour intervals, 1 to 6 blood samples before the experiment was started and 4 to 8 blood samples after giving either glucose alone or glucose plus insulin. In order to minimize the individual variability of the test animal several comparative experiments, first with sugar alone and then with sugar plus insulin, were performed on subsequent days on the same rabbit. In another group of experiments this was reversed so that first sugar plus insulin and then sugar alone was given to the same rabbit. The data are summarized in Table I. The figures represent the difference in sugar concentration between femoral artery and femoral vein that were observed at the time intervals noted after giving either glucose alone or glucose plus insulin. The figures of each vertical column represent one individual experiment. An average difference between arterial and venous blood sugar for each observation time

¹ Cori, Cori and Goltz, *J. Pharm. Exper. Therap.*, 1923, xxii, 355.

² Frank, Nothmann and Wagner, *Klin. Wochenschr.*, 1924, iii, 581.

SUGAR CONCENTRATION IN BLOOD DURING INSULIN ACTION 73

was calculated from the horizontal columns. It will be noted that the average differences for the series with glucose plus insulin are for all observation times greater than those of the series with glucose alone. This would mean that more sugar disappears from the blood into the muscles in the former series than in the latter, or that rabbits receiving insulin plus glucose show a larger intake of sugar by the muscles than rabbits receiving glucose alone.

TABLE I.

The differences between arterial and venous blood sugar that were observed 30, 90.....240 minutes after giving either glucose alone or glucose plus insulin are recorded and the average differences for each observation time are calculated.

Minutes	(A.) Glucose alone		(B.) Glucose plus Insulin	
	Difference F. A.—F. V.	Average difference F.A.—F.V.	Difference F. A.—F. V.	Average difference F.A.—F.V.
	mgm.	mgm.	mgm.	mgm.
30	9; 20; 20; 32; 20; 21;	20.3	18; 27; 23; 31; 27; 27; 27;	25.7
60	4; 11; 28; 18; 4; 7;	12.0	17; 24; 25; 25; 21; 16; 29;	22.4
90	18; 13; 14; 19; 15; 20;	16.5	17; 25; 21; 25; 25; 22; 19;	22.0
120	6; 27; 26; 19; 13;	18.2	23; 23; 20; 35; 29; 21; 24;	25.0
150	4; 6; 19; 20; 12;	12.2	7; 26; 19; 20; 27;	19.8
180	22; 11; 12; 13; 1;	11.8	24; 16; 22; 14; 18; 20;	19.0
210	3; 9; 15; 10;	9.2	20; 12; 9; 13; 20;	14.8
240	2; 5; 1;	2.7	19; 8;	13.5

36 (2559)

The insulin content of the pancreas and other tissues in animals
poisoned with phlorhizin.

By GERTY T. CORI.

[From the State Institute for the Study of Malignant Disease,
Buffalo, N. Y.]

The insulin content of the pancreas, liver, blood, and skeletal muscles of starved and completely phlorhizinized cats and of normal cats, starved for the same length of time has been determined. The Doisy, Somogyi and Shaffer process with only slight modifications was used for the preparation of insulin, and the insulin obtained was tested on mice. From organs other than the pancreas very small amounts of insulin were obtained. The yields have a comparative value only and have been expressed in mouse units, since it did not seem justified to calculate these very small amounts of insulin in the commonly used rabbit units. The amount of insulin injected intraperitoneally which lowers the blood sugar of a mouse (previously starved for 24 hours, weighing 18 to 22 gm. and kept at a room temperature not below 24° C.) in one hour to 0.038 to 0.044 per cent has been considered as a mouse unit. From the following table it may be seen that a lack of insulin production of the pancreas or a lack of insulin fixation in the tissues cannot account for the incapacity of phlorhizinized animals to metabolize sugar.

TABLE I.

The insulin content of the organs of control cats and of cats completely poisoned with phlorhizin, expressed in mouse units per gm. tissue.

	Phlorhizinized cats		Control cats	
	1	2	1	2
Pancreas	26	40	33	94
Liver	0.1	0.13	0.13	0.08
Blood	0.1	0.2	0.04	0.04
Muscle	traces (0.04)	traces (0.034)	traces (0.007)	traces (0.024)

SCIENTIFIC PROCEEDINGS.

New York Meeting.

Rockefeller Institute, November 19, 1924.

37 (2560)

Relation of vitamin A to growth, reproduction and longevity.

By H. C. SHERMAN and F. L. MacLEOD.

[From the Department of Chemistry, Columbia University, New York City.]

Experiments are described in which parallel groups of rats of identical previous history were fed upon two types of diet, one rather low and the other fairly high in vitamin A, from soon after weaning-time until natural death.

The smaller amount of vitamin A proved sufficient for normal growth up to nearly normal adult size, but not for successful reproduction, and rarely did it support satisfactory longevity. The parallel animals receiving the more liberal allowance of vitamin A grew to fully average adult size, were successful in reproduction and the rearing of young, and lived on the average a little over twice as long as those on the diet equally good in all other respects but lower in vitamin A.

These experiments show strikingly that a proportion of vitamin A in the food sufficient to support normal growth and maintain every appearance of good health, for a long time at least, may still be insufficient to meet the added nutritive demands of successful reproduction and lactation.

Along with the failure to reproduce successfully there usually also appeared in early adult life an increased susceptibility to infection and particularly a tendency to break down with lung disease at an age corresponding to that at which pulmonary tuberculosis so often develops in young men and women. The bacillus involved is different; but the close parallelism of increased sus-

ceptibility of the lung to infection at this stage of the life history appears very significant, especially in view of the fact recorded in another paper from this laboratory that the vitamin-A-content of lung tissue varies with that of the food.

Especially noteworthy was the repeated observation of young females growing normally and presenting every appearance of good health throughout youth on a diet low in vitamin A, but failing utterly to succeed in the rearing of a second generation, and showing a strong tendency to break down in health at an age at which they should have been in the prime of life.

Thus it is clearly shown that vitamin A is an even more important factor in the chemistry of food and nutrition than has previously been appreciated, for it must be supplied in liberal proportion not only during growth but in the food of the adult as well, if a good condition of nutrition and a high degree of health and vigor are to be maintained.

38 (2561)

Oil activated by irradiation. II. Separation into an antirachitic and an inactive fraction.

By ALFRED F. HESS, MILDRED WEINSTOCK, and DOROTHY HELMAN.

[From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.]

In previous communications^{1, 2} it has been reported by us that inert vegetable oils, such as linseed or cotton seed oil, can be endowed with antirachitic properties by means of irradiation with ultraviolet rays, and furthermore that the same property can be communicated to lettuce or to growing wheat by this form of radiation.

It is well-known that the antirachitic principle of cod liver oil is contained in its unsaponifiable fraction and that the saponifiable fraction is inert (Zucker). It seemed therefore of interest to

^{1, 2} Hess, A. F., and Weinstock, M., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 6, 5.

ascertain whether the activated vegetable oil could be separated into similar fractions. A preliminary test showed that the unsaponifiable fraction of cotton seed oil was unable to protect rats which were placed on a low phosphorus rickets-producing diet. the same was found to be true in respect to linseed oil. When, however, irradiated linseed oil was fractioned, it was found that 0.1 gm. of the unsaponifiable fraction was sufficient to confer protection. The criterion of its efficacy was the absence of microscopic lesions at the costochondral junctions. Furthermore the blood of these rats contained more than twice as much inorganic phosphorus as the blood of similar rats which had received the non-irradiated oil. It would seem that irradiation of the vegetable oil had produced a substance similar in its properties to that in cod liver oil.

39 (2562)

Rate of metabolism and sex determination in Cladocera.

By ARTHUR M. BANTA and L. A. BROWN.

[*From the Laboratory of the Station for Experimental Evolution,
Cold Spring Harbor, N. Y.*]

Cladocera reproduce mostly by parthenogenesis, and for long periods of time only female young may appear. Sometimes, however, males arise from parthenogenetic eggs, which eggs are indistinguishable from the parthenogenetic eggs ordinarily giving rise to females. At times sexual eggs appear and, if pairing occurs, these larger eggs pass into specially modified egg cases, the ephippia, within which they are cast off, undergo the earlier developmental stages, and enter a resting or latent period. This latent period is much prolonged (probably for months or even years with some forms) unless the eggs undergo desiccation or freezing or both. After desiccation or freezing if returned to water at 10° to 25° C. the sexual eggs develop into female young.

Experience in rearing several species of Cladocera lead the senior writer to conclude¹ that different environmental conditions are responsible for the occurrence of parthenogenetic females, or

¹ Banta, Arthur M., *PROC. SOC. EXP. BIOL. AND MED.*, 1916, xiv, 3.

of males, or of sexual eggs. In the collaborative work (most experiments were with *Moina macrocopa*) it was found that crowding the mothers caused the production of a variable percentage of males, while sisters given identical treatment, but uncrowded, produced only females. It was also found that CO₂ or uric acid treatments slightly increased the percentage of males in crowded or semi-crowded bottles. However by far the majority of treatments (including other excretory or related products), which altered the chemical condition of the medium in crowded or semi-crowded bottles, reduced male production. These experiments suggested the accumulation of excretory products as associated with male production.

Later, low temperature and chloretone were added to the list of treatments inducing male production. Adrenal cortex and alcohol (light dosages), on the other hand, eliminated or greatly reduced male production, whereas control bottles containing the same numbers of mothers produced characteristic male percentages.

The critical period at which the eggs in the ovary of a mother may be caused to develop either into females or males was found to be about two hours and to end about four hours before the egg leaves the ovary and passes into the brood chamber. This is approximately the time at which Kühn found that the maturation spindle is formed. Kühn² also found, as Weismann³ had done before him, that there is a single division without chromatic reduction in the maturation of the parthenogenetic egg. The presumption is strong that environmental conditions, in some manner, alter the chromatic behavior in the single maturation division and thus affect the sex of the forthcoming young Cladocera. According to Miss Taylor,⁴ however, the female and male have the same number of chromosomes (eight).

It was surmised and proven by direct observation and the accumulation of ample data that male production is associated with a lowered rate of metabolism of the mother during maturation of the eggs. This is true whether the male production is associated with crowding, low temperature, or any of the other conditions favoring male production. Hence it is assumed that the rate of metabolism, or some phenomenon associated with it, in

² Kühn, A., *Arch. Zellforsch.*, 1908, i, 1-50.

³ Weismann, A., *Zeit. wiss. Zool.*, 1876-1879, 27-33.

⁴ Taylor, M., *Zool. Anz.*, 1914, xlv, 21-24.

some manner alters the maturation division and thus determines the sex of the young. However it operates, we have found that lowered rate of metabolism is associated with male production in Cladocera; that agents which increase the rate of metabolism reduce or eliminate the occurrence of males; and that treatments which decrease the rate of metabolism induce the production of males. Further, within limits, the percentage of males produced is roughly proportional to the amount of retardation exhibited by the mothers.

Riddle⁵ has suggested that change in rate of metabolism may account for the results obtained by Whitney,⁶ Shull⁷ and others on control of sex in rotifers. It seems questionable whether the factor of *change* in rate of metabolism enters into the present case, if indeed it does in rotifers. It appears rather that a lower rate of metabolism is associated with male determination in the parthenogenetic eggs; and a higher rate of metabolism, such as is usual for ordinary laboratory cultures and in nature, is associated with female determination. It seems probable that also with rotifers, phylloxerans, aphids, paedogenetic diptera, and many other organisms, in which parthenogenesis alternates with gamogenesis, the production of males and the occurrence of sexual females are determined by rate of metabolism.

NOTE: Obreshkove, working in our laboratory, has found evidence that male Cladocera have a higher rate of metabolism than females, a fact previously known for the males of many forms, including man. In our present case *male causation* is associated with a lowered rate of metabolism at time of egg maturation *but the resultant males have the higher metabolic rate characteristic of males in general.*

⁵ Riddle, O., *Am. Nat.*, 1916, 1, 345-410.

⁶ Whitney, D. D., *J. Exp. Zool.*, 1914, xvii, 545-558.

⁷ Shull, A. F., and Ladoff, S., *J. Exp. Zool.*, 1916, xxi, 127-161.

40 (2563)

Electrical stimulation of luminescence in Ctenophores.

By A. R. MOORE.

[*From the Physiological Laboratory of Rutgers University, New Brunswick, N. J.*]

A dark adapted specimen of Mnemiopsis, when subjected to the action of the galvanic current, reacts by a display of luminescence at the anode. In lively specimens the glow may extend to the middle of the animal, and last for as long as five seconds. The instant the current is broken the glow at the anode ceases and a momentary flash occurs on the cathodal side of the animal. These effects are observable whether the current be passed through the animal lengthwise or transversely. If a transverse incision be made in a specimen of Mnemiopsis, then, when the current is passed longitudinally through the animal, in the region of the cut surfaces luminous secondary anodes or cathodes appear whenever the animal is subjected to the make or break of the current. The reaction of Mnemiopsis to the galvanic current therefore constitutes a complete case of "reversed Pflüger's law". The same phenomena may be demonstrated with specimens of Beroë, except that the effect of the break shock is not apparent in a luminescent flash at the cathode. In a solution of pure NaCl no galvanic stimulation of luminescence is possible, but in a solution containing 1 mol CaCl_2 to 500 NaCl galvanic stimulation occurs, although the luminescent flashes are of short duration. The current used was from 3 dry cells and from 2 to 10 milliamperes strength.

The fact that secondary regions of stimulation can be created by means of incision proves that the current produces excitation only at the protoplasm-sea-water surface, and that a movement of ions within the cells and their subsequent blocking at the cell boundaries is not in this case the cause of stimulation. This conclusion is further supported by experiments with the spark discharge from an influence machine. Electricity from such a source possesses a potential of several hundred volts and a current strength of less than $1/20$ milliampere. The spark discharge is known to have a high physiological efficiency for vertebrate tissues.¹ In order to test the effect of the spark on Mnemiopsis or

¹ Loeb, J., *Pflüger's Archiv.*, 1897, **lxix**, 99.

Beroe, the specimen was laid on a glass plate. In a moment the luminescence from mechanical stimulation ceased. The animal was now brought as near as possible to the spark discharge; no effect in a luminescent reaction was observed. If the arrangement of the experiment was altered by connecting one of the poles of the machine by means of a wire with the glass plate, then, by bringing the other terminal near the animal, a spark 1 or 2 cm. long could be played over the surface of the animal. The result in the case of Beroe was again negative—no stimulation of luminescence. With Mnemiopsis a slight stimulation occurred when the spark was applied.

For these reasons the question may be raised whether the mechanism of electrical stimulation of luminescence in Ctenophores does not differ from that of electrical stimulation of the neuromuscular system of higher forms.

41 (2564)

Further studies on so-called bacteriophage.

By J. BRONFENBRENNER

[From the Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.]

D'Herelle's hypothesis as to the parasitic nature of the "bacteriophage" is questioned by a number of investigators. However, the evidence which would definitely disprove its animate nature is still lacking. It seemed to us that the question of the nature of the bacteriophage might be approached by the study of its metabolism. The behavior of the bacteriophage with regard to respiration was first studied.

Different amounts of bacteriophage, representing from 5×10^{10} to 1×10^{15} active units, were placed in a microrespirometer, constructed on the general principle of the respirometer of Professor Osterhout, for varying periods of time up to 96 hours at 22°C . It was found that neither entire active filtrates nor the precipitated substance produce any CO_2 in the presence of oxygen. The method used was sensitive enough to indicate production of CO_2

under similar conditions by several viruses and bacteria, some of which had been kept in dry state for a period of years. In many instances, particularly in the case of fresh tissue emulsions and of bacteria dried over H_2SO_4 for 24 hours, CO_2 production could be detected 2 to 3 hours after placing minute amounts (0.001 gm.) of material in the apparatus.

42 (2565)

Blood reaction and respiration.

By D. D. VAN SLYKE, A. B. HASTINGS, L. D. MURRAY, and
H. W. DAVIES.

[From the Rockefeller Institute for Medical Research, New York City.]

Experimental and clinical observations indicate that when the respiratory mechanism is normal, increase in alkaline reserve is only partially compensated by increase in CO_2 tension, so that increase in pH also occurs. In the same way decrease in alkaline reserve is accompanied by decrease in pH. There is a decrease in CO_2 tension but not sufficient to prevent pH change. The usual percentage change in hydrion concentration is about twice that in CO_2 tension. The arterial CO_2 tension is kept normally between 35 to 45 mm., which is a much narrower range than would be necessitated for the maintenance of normal pH. The conception of the CO_2 tension as a factor physiologically important only from its relationship to blood pH is not consistent with these facts. When conditions force the organism to choose between change in CO_2 tension and change in pH it tends to compromise between the two, and acts in a manner to indicate that maintenance of normal CO_2 tension is in itself an important factor.

43 (2566)

The effect of washing on the resistance of erythrocytes to hypotonic hemolysis.

By S. C. BROOKS.

[From the Division of Pharmacology, Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.]

Brinkman and van Dam¹ claim to have shown that the resistance of rabbit erythrocytes to hypotonic hemolysis is increased by washing them in a physiologically balanced solution, while Snapper² publishes data tending to show that washing in 0.9 percent NaCl decreases their resistance. This difference is not necessarily a general one due to the chemical composition of the solutions, as appears from the following experiments:

Blood was taken aseptically by heart puncture from a dog and from four guinea pigs whose blood was pooled. The blood was defibrinated by shaking with fragmented glass. 1.5 cc. of each kind of cell was suspended in 15 cc. of Ringer-Locke's solution lacking phosphate and bicarbonate, and a like amount in 15 cc. of 0.9 percent NaCl. After about ten minutes these, together with samples of undiluted defibrinated blood, were centrifuged, and the supernatant liquid drawn off so as to leave the total volume 15 to 20 percent in excess of that of the cell sediment, which was then stirred up in this volume.

Portions of 0.05 cc. of each of the six lots were added to 2.5 cc. of each of several appropriate dilutions of 0.9 percent NaCl, centrifuged, the supernatant liquid decanted, and the sediment of cells hemolysed in 2.5 cc. of distilled water. The color of both the decantate and the hemolysed sediment was then measured by comparison with a Madsen scale and the amount of hemolysis in percent calculated from these data. The method of calculation and the necessary corrections have been described.³

Curves were drawn showing degree of hemolysis in percent plotted against concentration of NaCl, and the relative concentrations required to produce 20, 40, 60 and 80 percent hemolysis

¹ Brinkman, R., and van Dam, E., *Biochem. Zeitschr.*, 1920, cviii, 35-51.

² Snapper, J., *Biochem. Zeitschr.*, 1912, xliii, 266-74.

³ Brooks, S. C., *J. Med. Res.*, 1920, xli, 399-409.

determined graphically. The resistance may be considered to be the inverse of these concentrations.

Ringer-Locke's solution, as used, lowered the resistance to 99 and 98 percent for dog and guinea pig erythrocytes respectively. The loss of resistance due to washing in Ringer-Locke's solution is so small that it may be considered to be without significance. Washing in a balanced solution must be repeated at least twice, as in the experiments of Brinkman and van Dam, if any considerable effect is to be observed.

NaCl 0.9 percent increased the resistance of dog and guinea pig red blood cells 2 and 20 percent respectively, but so altered the shape of the titration curve that the quantitative significance of these figures is dubious. Some increase in resistance is quite clearly shown.

It is apparent from this that NaCl may under appropriate conditions increase the resistance of erythrocytes, and that therefore the decrease in resistance found by Snapper, and considered by him to have been due to NaCl was quite probably due to mechanical injury from repeated centrifugation (he centrifuged his cells four times). Such injury can be prevented by the addition to the wash fluid of 0.125 percent gelatine.⁴ It is also possible that the effects of NaCl vary according to the duration of its action, subsequent injury reversing a primary increase in resistance such as Brinkman and van Dam produced by the washing out of lecithin in a balanced solution.

⁴ Rous, Peyton, and J. R. Turner, *PROC. SOC. EXP. BIOL. AND MED.*, 1915, xii, 107-8.

44 (2567)

Comparison of reactions in individuals to toxins prepared from three strains of scarlet fever streptococci.

By MARY B. KIRKBRIDE and MARY W. WHEELER.

[From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.]

Twenty-two persons were tested with toxins from three different strains of streptococci isolated from cases of scarlet fever. Toxin No. 1 was prepared from a strain received from Doctor Dochez. Toxins Nos. 2 and 3 were obtained from Doctor Anna Williams. They were prepared from two different strains. Number 3 was from the Dick strain.

Fourteen persons gave similar reactions to all three toxins. Four gave very slight reactions to toxin No. 3 and no reactions to the other two. Three gave very slight reactions to toxin No. 2 and none to the other two.

The reactions in one individual were of exceptional interest. Toxins 2 and 3 failed to give any reaction, toxin 3 in two skin-test doses; toxin No. 1, however, gave a very marked reaction, even on repeated tests. The reaction to this toxin was apparently typical, commencing in 4 to 6 hours, reaching its maximum in about 24 hours and then fading rapidly. There was no indication of pseudo reaction—the test with heated toxin being negative. Moreover, toxin neutralized with antitoxic serum received from Doctor Dochez gave no reaction, although normal horse serum even in low dilutions failed to neutralize the toxin.

In tests on two other susceptible individuals the serum of this person, in a dilution of 1:4, neutralized both toxins 1 and 3, but the mixture of her serum with toxin No. 1 induced a definite reaction in the person herself. Toxin No. 2 was not tested.

45 (2568)

Reactions induced by intracutaneous injections of toxins of streptococci from scarlet fever.

By MARY B. KIRKBRIDE and MARY W. WHEELER.

[From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.]

With toxins prepared in this laboratory from a strain of a scarlet fever streptococcus received from Doctor Dochez, no skin reactions were induced in rats, mice, guinea-pigs, kittens, chickens, pigeons, monkeys, sheep or a calf. Questionable reactions were obtained in two young pigs with undiluted toxin and also in four rabbits out of eighteen, the remaining fourteen giving no reactions.

Definite skin reactions, however, have been induced in white goats. All fourteen goats tested gave reactions to the toxin, but individual goats appeared to vary in susceptibility. With ten, definite reactions were obtained with 0.2 cc. of a 1:500 dilution of a toxin which gives a reaction in a 1:1000 dilution in a susceptible human being. Four other goats gave reactions to lower dilutions, varying from 1:50 to 1:250. The reactions in goats reached a maximum in 18 to 24 hours and at this stage were usually quite similar to those observed in human beings. In the more susceptible goats according to the potency of the material tested, these reactions varied in degree from slightly reddish areas, 1.5-2 cm. in diameter with no swelling, to large reddish areas from 3-5 cm. in diameter with considerable swelling. All reactions, even the most severe, faded in 48 to 72 hours. With some goats, the darker color of the skin made the readings difficult.

Toxin heated for one hour at 100° C. and uninoculated broth gave no reaction nor was any obtained with toxin neutralized with scarlet fever antitoxic serum, but normal horse serum, even in low dilutions did not neutralize the toxin. Toxins from another strain of streptococcus from scarlet fever, which gave no reaction in susceptible human beings in a 1:250 dilution, also induced no reaction in goats, in the same dilution.

Since comparative tests with different toxins indicated that there is a definite relation between skin-test dose of these

toxins for man and for the goat, and since repeated injections of toxin do not appear to interfere with the reaction, these animals should prove of value in the preliminary standardization of the toxin—tests which up to the present have been made on persons.

On the other hand, in attempts to use these animals for standardizing antitoxic horse serums, it was found that after repeated intracutaneous injections, the goats had become so hypersensitive to the foreign protein that further injections resulted in marked local swelling and oedema. Hence, it would probably be impossible to use the same animal for this purpose more than once or twice.

46 (2569)

The cyanosis of peripheral venous engorgement.

By SAMUEL GOLDSCHMIDT and ARTHUR B. LIGHT.

[*From Department of Physiology, University of Pennsylvania Medical School, Philadelphia, Pa.*]

When the arm is hung down at the side of the body the veins become markedly engorged. Also, there develops a dusky bluish color of the skin of the hand and wrist, extending for a distance up the forearm. Such a state of affairs has usually been assumed to be due to stasis of the blood in the arm.

If the volume flow of blood through the arm is decreased, after a sufficient interval of time, assuming that metabolism progresses normally, there must be a point where the venous oxygen unsaturation is increased above the normal.

In studies using blood taken from the veins on the dorsal surface of the hand, we have found that there is usually a significant decrease of oxygen unsaturation of this blood, when the arm is hung down and immobilized. In eleven experiments, there is a significant decrease of the oxygen unsaturation in six. Of the five remaining, the unsaturation is the same in two cases and decreased in three. In addition, the oxygen capacity of the blood, under these conditions, is usually either increased or remains the same. Of thirteen experiments the capacity is increased in four cases, remains the same in seven and is decreased in two.

It is obvious that such results cannot be explained on the assumption of a simple stasis. The engorgement of the veins suggests that this engorgement extends for some distance towards the arterial side of the vascular bed, at least, into those vessels, venules and capillaries, which are responsible for skin color. Once these vessels have dilated to their maximum degree, there would be a larger volume of blood available for gaseous interchange, which with a normally progressing metabolism would result in less oxygen extracted from each unit volume of blood to meet tissue requirements. However, if there is a decrease in volume flow, the condition in respect to oxygen would be but a temporary one. We have found a decreased oxygen unsaturation after the arm has been held down for an hour or longer. This suggests that the volume flow of blood is not decreased. Only one explanation can be offered, namely, that there is an increase of arterial inflow of a sufficient degree to overcome the lack of the venous pump mechanism and the increased hydrostatic pressure which must be overcome in returning the blood to the heart.

The bluish dusky appearance of the skin is thus not associated with increased oxygen unsaturation of the blood. We have evidence against the assumption that the blood in the superficial skin vessels is different from that which we obtain from the veins. It would seem then that when the vessels of the skin, which give it color, are engorged in large number with blood of normal or even higher than normal blood oxygen, the skin takes on a bluish tinge. The blood coursing through the skin vessels does not appear blue under ordinary conditions, because the vessels are in a relative state of constriction. When, however, they become dilated and the blood is distributed more uniformly over a wide area, the blood imparts a dusky bluish color to the skin. This color appears well below the threshold value for oxygen unsaturation at which cyanosis has been said to appear. The increased amount of hemoglobin, when it occurs, may contribute to this phenomenon. On the other hand, we have noted marked blueness of the skin in the absence of increased hemoglobin.

The concentration of the blood can be explained by capillary and venule dilatation. This brings about an increased area for filtration of fluid from the blood, which is aided by an increase in filtration pressure. The latter is due to a general increase of pressure in these vessels. In the experiments in which concentra-

tion does not occur, or when blood shows a tendency to dilution, we may postulate variations of balance between blood flow and the increased pressure in the vessels.

47 (2570)

Cinematography of skin capillaries in the living human subject.

By ALFRED E. COHN, J. HAMILTON CRAWFORD and
H. ROSENBERGER.

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

Lombard¹ in 1911 showed that by illuminating the skin, the capillaries could be seen under the microscope. Many observations have since been carried out on the changes which take place in various diseased conditions. The possibility of studying alterations in the capillaries by means of cinematography first suggested itself to Krogh and Rehberg.² They developed a method of taking pictures of the circulation in the capillaries in the tissues of *Rana temporaria*. It is naturally simpler to obtain records from the tissues of frogs than from human tissues since for this purpose a technique resembling that used in histological photomicrography suffices. In the human case transmitted light cannot be used. The method of illumination from above has therefore been universally adopted. This method is difficult because, by it, contrast and intensity of light are much reduced as compared to these qualities obtained by transmitted light. Weiss,³ in 1916, devised an apparatus for photographing human capillaries, and published photographs obtained by his method in various diseased conditions. He used indirect illumination and required an exposure of one-quarter to three-quarters of a second to obtain pictures. Siedentopf (Zeiss) likewise has devised an apparatus for the instantaneous photography of skin capillaries under normal

¹ Lombard, *Am. J. Physiol.*, 1911-12, xxix, 335.

² Krogh and Rehberg, *Am. J. Physiol.*, 1924, lxxviii, 153.

³ Weiss, E., *Deutsch. Arch. f. klin. Med.*, 1916, cxix, 1.

conditions. In this apparatus also indirect illumination is used. Sheard⁴ has reported the fact that he has been able to make photographs in rapid succession, but not cinematographic, of skin capillaries in the living human body.

During the past year experiments have been continuously in progress in the Hospital of the Rockefeller Institute having as their object the photography, and more especially the cinematography, of human capillaries. In July, 1924, the first cinematographic records were made by us. At first we also used indirect illumination and by this means obtained photographs. These, we thought, were not sufficiently satisfactory for our purpose. Since then we have improved our apparatus and can now obtain fairly satisfactory cinematographic pictures.

Our apparatus in its present form consists of four parts: (1) a lighting system, (2) the microscope, (3) a stand for holding and adjusting the finger, and (4) the camera.

(1) As our source of illumination we use a 15 ampere direct current arc lamp with special carbons. The light from this passes through a series of heat and light filters, and also through a powerful condensing system, so that the cool light is focussed on a small point. We have introduced a new feature in capillary microscopy, namely, direct illumination. The point of light enters the system for direct illumination and also passes through a polarizer. The latter has been introduced to prevent the reflection of light from the surface of the oil on the finger, which takes place with direct illumination and prevents the capillaries from being seen. With this illumination we have been able to obtain detail in our pictures to a degree which was impossible with the indirect method.

(2) In the microscopic system we use a 16 mm. apochromatic objective with a 5x (Leitz) eye piece. This combination, which gives a magnification of fifty, has so far proved the most satisfactory arrangement. The tube of the microscope is kept fixed and all focussing, except fine adjustment, is managed by moving the stage of the microscope. The tube of the microscope fits accurately into the camera.

(3) The stand for the finger has been constructed so that it is part of the stage of the microscope. The patient's finger is placed in a holder and fixed—without in any way interfering with

⁴ Sheard, *Science*, 1924, lx, 409.

the circulation of the finger—so as to avoid independent movement. By means of certain adjustments on the stand, the finger can be moved to any desired position.

(4) The camera was devised for us especially for this purpose. The film is driven by a motor, and the number of exposures a second is indicated by a special speedometer recorded on the film.

With the apparatus in its present form satisfactory cinematographic photographs can, as has been said, be taken. A film taken by our method which showed the corpuscles in motion was demonstrated to the meeting. The detailed description will be published later.

48 (2571)

Twelve per cent dextrose media for prolonged anaerobe growth.

By WILLIAM N. BERG.

[*From the Berg Biological Laboratory, Brooklyn, N. Y.*]

The following easily prepared media permits the vigorous growth of several strains of a typical anaerobe, the bacillus of symptomatic anthrax (*B. gangraenae emphysematosae*, or *B. chauveii*) for 30 or more days.

Composition of media :

water	7000 cc.
liver, hog	2500 gm.
peptone	80 gm.
Ringer salt	40 gm.
chalk	350 gm.
dextrose	1000 gm.

The above quantities are for one 12 liter flask. It is essential that the liver be not more than 24 hours old, and should be obtained at the abattoir. The media is sterilized by placing the flasks or jars in the autoclave, bringing the steam pressure up to 15 lbs., and maintaining it at 15 lbs. for 45 minutes. The media is not injured by this severe heat treatment. Several lots

were autoclaved three times without interfering with the ability of the media to start and maintain the growth of the bacillus of symptomatic anthrax.

After autoclaving let the media cool, incubate for 24 to 48 hours, and the media is ready for inoculation. No titration or pH adjustment is necessary. The media keeps well; small jars containing a deep layer of it were good after having been kept at room temperature for several months.

Just why this media permits so much longer growth than others can not be stated with certainty. It is probably due in part to the high concentration of dextrose, and the presence of the calcium carbonate which neutralizes the butyric acid formed by the bacillus. That this neutralization is not complete is shown by the acid reaction of the culture filtrate, which has a pH of 5.2 to 5.8.

49 (2572)

Studies on the action of insulin neutralized with alkaline solutions.

By E. F. MUELLER, M.D., and C. N. MYERS, Ph.D.

[From the Department of Dermatology and Syphilology, College of Physicians and Surgeons, Columbia University, New York City.]

It was observed that the result of insulin injections varied with the route of administration. Using normal rabbits for our experiments, it was determined that if the injection was given intradermally the effect was more lasting than if an equal amount had been injected subcutaneously. These findings, the subject of an earlier paper, led to the question as to whether or not similar results can be obtained in the human body. Before proceeding with our studies it was found necessary to conduct comprehensive preliminary tests in animals, the results of which were important biologically and clinically. The following problems presented themselves:

(1) What pathologic changes does the intradermal injection of insulin cause in the skin?

(2) Are the changes following the intradermal injection of neutralized insulin of a different character?

(3) Is neutralized insulin less effective than the original preparation?

The tests which were to form the basis for the answer to the first two questions were made with intradermal and subcutaneous injections into normal rabbits and rats of the original preparation (iletin). The readings made at the end of $\frac{1}{2}$, 1, and $1\frac{1}{2}$ hour periods coincide with the observations of D. J. Bowie and W. L. Robinson.¹

Oedema and light degenerative processes were found especially in the tissue surrounding the subcutaneous vessels as a result of giving insulin intradermally as well as subcutaneously. Furthermore, degenerative processes in the walls of the vessels were determined, with beginning of complete thrombosis in these vessels. Some vessels showed complete thrombosis and complete destruction of greater parts of their walls. Beside these very marked pathological findings at the site of the involved vessels, there were observed degenerative changes, and even a small number of leucocytes and round cells in the surrounding tissues. The vessels not particularly injured were dilated, and contained more white cells than usual. In the epidermis special pathologic changes were missing. The changes in the subcutaneous tissues mentioned above were more marked after subcutaneous than after intradermal injections.

In a second series of similar tests in rabbits and rats, insulin was injected intradermally and subcutaneously after adding an equal amount of 1 percent sodium bicarbonate solution immediately before injection. The results of the histologic examination of these specimens were quite different. No thrombosis, no degeneration in the walls of the vessels were found, but the vessels were dilated. Small numbers of cells were found in these areas. No pathologic tissue changes were observed.

In a third series of animals injected with a solution of acetic acid of different strength, similar lesions were observed as in the first series, representing findings after the normal insulin injection.

These three series of animal tests will be discussed in detail

¹ Bowie, D. J., and Robinson, W. L., *J. Lab. and Clin. Med.*, 1923, viii, 5.

in a later paper, but it may be concluded that the main reason for the pathologic changes in the vicinity of the insulin deposit must be the small acid content of the insulin preparation which usually contains acetic acid.

The last question as to how insulin acts if neutralized immediately before injection was answered by two series of tests in normal rabbits. It was found that neutralization of insulin by sodium bicarbonate (1 percent solution) did not affect the insulin action at all. Intradermal as well as subcutaneous administration of neutral insulin did not show any decrease in its effect upon the blood sugar content. The curves of sugar determination in certain intervals were as low as those expected from the use of the original preparation.

A particular observation may be mentioned, namely that administration of neutralized insulin in some instances is followed by a greater effect than a like dose of the original acid agent. From further studies we assume that this phenomenon may be caused by the dilution of the drug. Insulin diluted by distilled water has in some instances a similar increased action.

From the result of the above studies it has been concluded that certain pathologic tissue changes in the vicinity of insulin deposits are caused by the acid content of the insulin preparation (iletin). Practically they are not very marked. They can be avoided by neutralizing insulin immediately before injection. This neutralization does not diminish the action of insulin upon the blood sugar content.

50 (2573)

Biochemical studies on the behavior of the leucocytes after intravenous administration of alkalized salvarsan.

By E. F. MUELLER, M.D., and C. N. MYERS, Ph.D.

[*From the Department of Dermatology and Syphilology, College of Physicians and Surgeons, Columbia University, New York City.*]

I.

The reaction of the involuntary nervous system to intravenous injection of salvarsan, neosalvarsan and silver salvarsan, and the influence of this reaction on the neutrophile leucocytes have been discussed in two previous articles.¹

It was found that in humans the number of white blood cells decreases for a short period immediately following an intravenous injection of salvarsan. This sudden decrease is prolonged in patients who present manifestations of the so-called nitritoid crisis. An intravenous injection of silver salvarsan, however, is not followed by a similar reaction.

These findings, which were discussed at length in the foregoing articles, led to a study of the details of this leucocytic reaction, the main problem being: Where are the leucocytes during the period of their absence from the periphery? It seemed probable that they had migrated to the inner organs. But no proof of this theory could be elicited from the human body, as it is impossible to examine intimately the inner organs of human beings after salvarsan injections except in rare instances, and never in a sufficiently large number of instances to warrant definite conclusions. Furthermore, it is known that abdominal operations, as well as general narcosis, are in themselves productive of a similar decrease in the number of leucocytes in the peripheral vessels. The only avenue of approach to a solution of this problem, therefore, was a study of the conditions resulting in the animal body.

II.

Animal experiments along these lines necessitated preliminary comparative tests. In different series of animal tests a similar decrease in the number of leucocytes was observed after the

¹*Arch. Derm. and Syph.*, 1924, x, 316, 606.

intravenous injection of salvarsan. The decrease is less pronounced or is entirely absent when silver salvarsan is administered.

The object of this series of tests was accomplished by determining the number of white blood cells in the heart blood. The heart blood for the count was obtained by puncturing the heart of the animal. It was observed that there was a practical correspondence in the number of leucocytes ascertained in blood from the peripheral vessels, from the tail vein and from the heart in the majority of animals, both before and after they had received salvarsan injections. From these experiments it was evident that the missing leucocytes were displaced from the peripheral vessels as well as from the heart and the main arteries, but it could not be determined whether they had disappeared during the period of peripheral leucopenia.

Further preliminary tests with normal rats were necessary to determine the leucocytic count in different organs after killing a normal animal. It was observed that immediately after death the blood count, made with heart blood, revealed remarkably lower values than those obtained before death occurred. The number of leucocytes found in the blood of the liver at the same time was very high; for instance 6,000 leucocytes in the heart blood corresponded to 12,000 or more in the liver. Different methods of killing the rats did not succeed in eliminating this factor of leucocyte displacement which, to a certain extent, resembled the change in the leucocyte count after the intravenous injection of salvarsan.

The only possible conclusion was that the act of killing by different methods produces a similar reaction on the leucocytes, and that this reaction in turn produces the displacement from the periphery and the heart to the liver. A continuation of the investigation of this problem along the lines of our former studies was not possible until this reaction could be avoided.

It may be interesting to mention that in rats dying with convulsions, the difference between the cell count in the heart blood and in the blood of the liver was more marked than in those animals in whom few or no convulsions were observed.

III.

When we succeeded in finding a method to kill a rat quickly enough for experimental purposes without producing such sud-

den reaction on the part of the animal as might involve a measurable change in the blood count, the elimination of the above mentioned reaction at the time of death became a reality. It is known that the intravenous injection of air is fatal, due to embolism of the pulmonary arteries. When killing a rat by this method practically no difference is observed between the cell count of the inner organs, especially of the liver and of the heart. The injection of 0.2 to 0.3 cc. of air into the vein of the leg was found sufficient to cause death without any measurable organic reaction. Based upon this knowledge, it was possible to begin a new series of tests to help locate the leucocytes missing after salvarsan injections.

The usual dose of salvarsan—90 to 180 mg. per kilo body weight—was given intravenously in the usual way; 0.3 cc. of air was injected either immediately or 2 minutes after the salvarsan administration. No appreciable difference in the number of white blood cells, both in the heart and in the liver, either before or after injection, was found in the animals injected with air only. The rats, which had received a salvarsan injection before death was induced by embolism, showed the following: There was a marked difference between the first count and the later findings in the blood of the heart and of the liver. The following example will demonstrate:

11:20 o'clock, blood from the tail.....	13600	leucocytes
11:25, salvarsan, 180 mg./kg. body weight, 2 percent solution.		
11:26, blood from the tail	9600	“
11:26.40, blood from the heart	10000	“
11:26.50, 0.3 cc. air intravenously.		
11:27, dead.		
11:30, blood from the heart	6800	“
	8400	“
blood from the liver	14200	“
	13000	“

From this and many similar tests it appears that the leucocytes, disappearing for a short period from the peripheral vessels, are retained in the liver for the time being. Our findings do not, however, allow of a conclusion as to whether or not other organs, such as the spleen, participate in this retention of the missing leucocytes, for it is not possible to determine the cell count of the spleen and of other areas of the abdominal circulation with sufficient accuracy to warrant definite conclusions. It is furthermore not always possible to apply the results of animal experimentation to the human economy; but considering the similarity of the

phenomena observed in the human and in the animal, the following can be asseverated.

After salvarsan injections in animals there ensues a peripheral leucopenia of short duration, during which period the leucocytes are mechanically retained in the blood of the liver. A short leucopenia after salvarsan injection is also observed in the human. It is highly probable that the leucopenia in the human observed after injection of salvarsan is also due to a similar retention of the leucocytes in the blood of the liver.

51 (2574)

On the antigens of red blood corpuscles.

By K. LANDSTEINER and J. VAN DER SCHEER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Experiments reported in a previous paper¹ led to the view that specificity as manifested by precipitin reactions on serum proteins and agglutinin or lysin reactions on red cells is based on structures of two different sorts. While the differences are apparent in the case of heterogenetic lysins and isoagglutinins, the findings in agglutinins and lysins in general seem to indicate the existence in the antigens of substances other than proteins (but probably bound to them).

As a matter of fact, the antigenic properties of blood cells have been ascribed to "lipoid" substances by several authors since the work of Bang and Forssman.² It was difficult to accept this notion in view of the fact that extracts of erythrocytes made with organic solvents and supposed to contain the antigen have a feeble action as compared with the original material, and that the residues after such extractions are still active (Thiele and Embleton).

¹ *J. Exp. Med.*, 1924, xl, 91.

² *Beitr. z. chem. Phys. u. Path.*, 1906, viii, 238. The bibliography will be given in the more detailed article on this subject. Cf. Schmidt, H., *Zur Biologie der Lipide*, Leipzig, Kabitzch, 1922.

We have studied the antigenic properties of the alcoholic extracts of horse blood cells, using the method employed by one of us for the heterogenetic haptene.³ By injecting mixtures of alcoholic extracts of horse red cells and diluted pig serum, we succeeded in obtaining hemolytic immune sera for horse blood stronger than those obtained with extracts alone. These sera, prepared by injections of extracts plus serum, or alone, differ markedly from those produced in the usual way by injecting unchanged erythrocytes. They exhibit a relatively higher ratio of lysin:agglutinin.⁴ They are distinctly inhibited by alcoholic extracts of horse blood, in this respect simulating heterogenetic antibodies. Their hemolysins were completely absorbed by donkey blood, contrary to the behavior of the common hemolysins.

After several extractions with boiling alcohol of horse blood stromata, previously treated with 5 percent salt solution and weak acid, the remaining substance still had antigenic properties. The resulting agglutinins were weaker than but similar to those with unchanged blood cells. This residue consisted of an insoluble substance remarkably resistant to acids and alkalies. It gave protein reactions, but also a positive test with orcin and copper sulphate.⁵ The nitrogen content of this product (about 13 percent) increased gradually when further extractions with alcohol were made. The orcin test was still positive.

These studies are being continued.

³ *Biochem. Ztschr.*, 1921, cxix, 306. *Proc. Acad. Sc. Amsterdam*, 1922, xxiv, 237. Landsteiner, K., and Simms, S., *J. Exp. Med.*, 1923, xxxviii, 127.

⁴ Landsteiner, K., and Prasek, *Zeit. f. Immun.*, 1912, xiii, 403.

⁵ Levene's Reaction.

52 (2575)

On individual differences of the blood of chickens and ducks.

By K. LANDSTEINER and C. PHILIP MILLER, JR.

[From the Laboratories of the Rockefeller Institute for Medical Research, New York City.]

It was originally demonstrated¹ in the case of human blood that there exist differences between members of the same species detectable by the isohemagglutinin reaction. Similar results have been found in several species of animals, *e. g.*, cattle, horses, sheep, dogs. With the blood of other animals, however, clear-cut reactions have not been obtained, although biochemical individual differences must for other reasons be assumed to exist. Adequate study of the question of isoagglutination has been hampered to a certain degree by the failure to obtain satisfactory results in common laboratory animals such as rabbits, rats, and mice.² It was our purpose to find suitable laboratory animals for further investigation, especially for breeding experiments (*cf.* von Dungern and Hirschfeld, Epstein and Ottenberg, and others) and for the study of racial differences.

Our observations were made on chickens and ducks. In most of the studies hitherto reported, isoagglutination (or heteroagglutination) by normal sera and isolysin reactions by immune sera have been employed. In our experiments we made use of immune sera obtained from a distant species. This method, which offers some advantages, can be used if by previous treatment with cells of individual bloods those agglutinins are removed which act indiscriminately upon all bloods of the species.³

Hadda and Rosenthal⁴ make brief mention of finding isolysins in chicken blood without presenting detailed information. Bailey⁵ failed to detect isoagglutinins in chickens.

¹ *Cent. f. Bakt.*, 1900, xxvii, 357. *Wien. klin. Woch.*, 1901, p. 1132. The experiments of Ehrlich and Morgenroth on goats.

² See Snyder, L. H., *J. Immunol.*, 1924, ix, 45.

³ Landsteiner, K., *Wien. klin. Rundschau*, 1902, xl. Hooker, S. B., and Anderson, L., *J. Immunol.*, 1921, vi, 419. Landsteiner, K., and van der Scheer, J., *J. Immunol.*, 1924, ix, 213, 221. Guthrie and Huck.

⁴ Hadda, S., and Rosenthal, S., *Zeitschr. f. Immun.*, 1913, xvi, 536.

⁵ Bailey, C. E., *Am. J. Hyg.*, 1923, iii, 370.

Our tests were made with anti-chicken and anti-duck sera obtained from rabbits injected with washed blood of Plymouth Rock chickens and domestic Mallard ducks, respectively. The following experiment is given as an example. The anti-chicken immune serum agglutinated chicken cells in a dilution of 1:1,200 (0.5 cc. diluted serum + 1 drop 5 percent washed blood cells). To each portion of 4 cc. of the serum, diluted 1:15, were added 2 cc. of washed blood sediment of each chicken to be tested.

TABLE I.
Reading after 2 hours at room temperature.

Absorbed sera	Blood cells.									
	1	2	3	4	5	6	7	8	9	10
1	0	0	±	0	+±	0	0	0	0	+
3	0	0	0	0	0	0	0	0	0	0
4	+	+	+±	0	+±	++	+±	+	+±	++
5	0	0	0	0	0	0	0	0	0	0
6	0	tr?	+	0	+	0	0	0	0	tr.
7	+	+±	++	0	+±	+±	0	0	±	++
8	±	+±	+±	0	+±	+	0	0	±	++
9	0	0	+	0	+	tr?	0	0	0	tr.
10	+	+±	++	+±	++	+	++	+	++	0

TABLE II.
Reading after night in ice-box and then one hour at room temperature.

Absorbed sera	Blood cells.									
	1	2	3	4	5	6	7	8	9	10
1	0	±	++	tr?	++	+	±	f. tr.	±	++
3	0	0	0	0	0	0	0	0	0	0
4	+±	++	+±	0	+±	++	+±	+	+±	++
5	tr.	tr.	0	0	0	0	0	0	0	0
6	tr.	+	+±	+	+±	0	+	±	+	+
7	+±	++	++	±	++	++	0	0	+±	++
8	+±	++	++	±	++	++	tr?	0	+±	++
9	+	+	+±	f. tr.	+±	+	f. tr.	0	0	+
10	++	++	++	++	++	++	++	++	++	0

Chickens

1 and 2. White Leghorn.

3, 4, and 5. Plymouth Rock

6. Brahma female.

7 and 8. Malay male and female. } Purchased from poultry breeder
9 and 10. Cochin male and female } as pure-bred chickens.

++ Large clumps seen macroscopically.

+ Small clumps seen macroscopically.

± Clumps seen microscopically.

tr. Trace.

f. tr. Faint trace.

These quantities proved adequate to remove all agglutinins acting on the blood used for absorption. After standing for one hour at room temperature, and over night in the ice-box, the separated liquid was tested for agglutinins against the various blood samples (0.3 cc. of the fluid + 1 drop 5 percent blood suspension).

The tests show the existence of marked differences between the blood corpuscles of the various chickens. In no case did agglutination take place with that blood with which the absorption was made. The reactions display a great variety since, according to the first reading (Table I.), only four pairs of bloods reacted identically or nearly so; and only two pairs, each belonging to the same race (or family?) reacted identically according to the second reading (Table II.). This would correspond to eight different types among the ten specimens examined. The fact that the examination of additional chickens revealed more differences suggests the existence of a still greater multiplicity. These observations agree well with the views on biochemical individuality resulting from the work on grafting of skin and other tissues, both normal and neoplastic (cf. L. Loeb⁶) and recall the findings of Todd and White⁷ on isolymins in cattle.

In conformity with the results of von Dungern and Hirschfeld, L. and H. Hirschfeld, and their co-workers, the reactions are not dependent on the race of the animal in such a way that a certain race is associated with a certain serological type. It is quite possible, however, that certain types of chicken blood or certain of the factors underlying the reactions show an unequal racial distribution, as pointed out by the workers just mentioned.

Isoagglutinin tests with chicken blood gave positive reactions of moderate intensity in some instances.

Tests on a small number of duck bloods yielded results similar to those on chickens.

Some tests made on white, hooded, and wild rats with hetero-agglutinins seem to indicate differences similar to those found in chickens, especially when different varieties were compared, but the results require confirmation.

⁶ Loeb, L., *The Amer. Naturalist*, 1920, liv, 45, 55.

⁷ Todd and White, *J. Hygiene*, 1910, x, 185.

53 (2576)

The effect of double adrenalectomy on the development of rickets in rats.

By ALFRED F. HESS and H. L. JAFFE.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.*]

It has been claimed by many investigators that there is an intimate association between the action of the adrenal glands and the development of rickets. Adrenalin has been given in this disorder by Stoeltzner¹ and "hormones" from the adrenal glands by Vollmer² and others who have claimed for them an important rôle in the pathogenesis and therapy of rickets. With these results in mind, we extirpated the adrenal glands in a considerable number of rats weighing from 100 to 150 gms. Complete bilateral adrenalectomy was performed by the lumbar route. These rats were then placed on the Sherman-Pappenheimer low phosphorus diet, or on a similar ration in which 5 percent of dried milk (Dryco) is substituted for an equal percentage of the flour. The latter is a much less defective ration than the former, and more suited for the nutrition of rats which have undergone a severe operation and evince a markedly lowered resistance.

No difference in regard to the development of rickets was found between the rats in which the adrenals had been removed and the control animals which had not been operated upon. Both groups regularly developed rickets of about the same degree as judged by the roentgenologic picture, the microscopic examination of the bones, and the percentage of inorganic phosphorus in the blood after a period of 26 to 42 days. We are of the opinion, therefore, that the functional activity of the adrenal glands is not an essential or important factor in the pathogenesis or cure of rickets.

¹ Stoeltzner, W., *Muench med. Woch.*, 1921, Nr. 46, 1481.

² Vollmer, H., *Jahrb. f. Kinderh.*, 1922, 1c, 133.

54 (2577)

Inheritance of an abnormality of form in *Paramecium aurelia*.

By J. A. DAWSON. (Introduced by L. L. Woodruff).

[*From the Zoological Laboratory, Harvard University.*]

Previous studies of abnormality of form arising from unknown causes in laboratory cultures of *Paramecium* have tended to show that such abnormality is not inherited, *i. e.*, at the time of fission only one daughter cell receives the abnormality. Thus, Jennings¹ found, in a *Paramecium* which developed a spine, that only one of the daughter cells received this modification, and that animals with a truncated anterior end transmitted the truncated condition to only one of the progeny. He also found that the truncated condition was lost after a few divisions, and all of the descendants became apparently normal. Stocking² confirms the observations of Jennings in regard to the non-inheritance of abnormality of form.

On February 23rd, 1924, the author found, in a laboratory culture of *Paramecium aurelia*, a number of peculiarly truncated specimens. The truncation was so pronounced that the animals were almost exactly one half the length of a normal *Paramecium*, and it was only after careful study that it could be identified as belonging to this genus. One hundred and thirty of these truncated individuals were isolated during the next three weeks and pedigree cultures of the progeny were kept for varying lengths of time. The culture medium consisted of timothy hay and whole wheat boiled for five minutes in spring or pond water. Standard quantities of these substances were used in making up each lot of culture medium.

Stated briefly the results of these breeding experiments with abnormal (truncated) *Paramecia* were: (1) Individuals remained in the truncated condition without dividing until death occurred. The maximum recorded period of life without fission in these individuals was thirty-three days. (2) Individuals

¹ Jennings, H. S., Heredity, Variation and Evolution in Protozoa, *J. Exp. Zool.*, 1908, v, 577.

² Stocking, Ruth J., Variation and inheritance in abnormalities occurring after conjugation in *Paramecium caudatum*. *J. Exp. Zool.*, 1915, xix, 387.

became apparently normal in all respects, or divided normally by fission to give apparently normal progeny. (3) Individuals produced from the anterior end, and occasionally from the posterior end, abnormal daughter cells. Considerable variation was shown in this abnormal progeny which either gradually became apparently normal, or increased in abnormality, and finally died. (4) Individuals produced a race which shows a definite though much less prominent truncation. This may perhaps best be described as a "notched" condition. To the present date (Oct. 9) this race has remained abnormal for one hundred and ninety-six generations, and pedigrees have been kept of eighty abnormal animals at one time, all of which have been derived from one ancestor (number five of those originally isolated).

For the past eighty days (July 13 to October 9) two pedigree cultures have been carried, consisting of five lines each of "notched" *Paramecia*, begun from an abnormal or "notched" descendant of the original truncated *Paramecium* in the 112th generation. The average division rate of each culture is similar to that of the earlier pedigree lines approximating very closely to one daily division. Although there is a slight tendency for the "notched" condition to disappear thus giving apparently normal offspring, a very high percentage of abnormality is shown in each line of both cultures.

A study of the division rate curve and of stained individuals indicates that endomixis occurs in this strain of *Paramecium aurelia*. So far as the present study has gone, no increase nor decrease in abnormality can be noted either before or after these endomictic periods.

Cytological study of both abnormals and apparent normals shows a typical macronuclear and micronuclear structure in all cases. Other details of structure, with the exception of the abnormality of form under discussion, are similar to those found in normal strains of *Paramecium aurelia*.

Both in mass cultures of stock animals made (a) from apparently normal animals and (b) abnormals, specimens similar to the original markedly truncated ancestor have been and are now being obtained.

Apparent normals arising from these abnormal lines have been carried in pedigree cultures on two different culture media,

and have so far given only apparent normals. There is no marked difference in the division rate of apparent normals and of abnormals.

Further experiments on the heritability of this abnormality of form are now in progress.

55 (2578)

The production of certain distinct types of reactions by the use of ovarian extracts.¹

By GEORGE N. PAPANICOLAOU.

[From the Laboratory of Cornell University Medical College, New York City.]

The present status of our knowledge on the rôle of the ovary as an endocrine gland, and on the exact physiological effects produced by the use of ovarian preparations and extracts, is far from being satisfactory. Though the importance of the ovary as an internal gland is well recognized, there is yet much controversy regarding the interpretation of its particular functions. Some investigators consider the *corpus luteum* as the only or chief active endocrine part of the ovary, while others attribute more importance to the interstitial tissue or to the follicular complex. This divergence of opinions is partly due to the fact that the methods used for the study of this problem have been somewhat incomplete and the conclusions drawn more or less exclusive. The *corpus luteum*, for instance, has been repeatedly underestimated in its importance as an endocrine gland since no positive results have been obtained by certain investigators, who have tried several luteal preparations. The fact that one type of ovarian extract may be inactive in a case where another is active must not be taken as a proof that the former does not possess a certain hormonal action which might be detected by the use of other methods. The ovary is a complex gland and possibly secretes more than one active substance. Such a view is well

¹ Aided by grant from Committee for Research on Sex Problems of National Research Council. Grant directed by Dr. C. R. Stockard.

supported by a large number of facts. The great diversity of the phenomena characterizing the sexual functions of the female could hardly be the result of a single factor or hormone. The structural complexity of the ovary and its diversity at different developmental stages, with the presence in it of several types of cells seemingly secreting actively in an alternating fashion, are also indicative of a multiple secretory function.

Such views as the above seem to be justified by some experiments carried out in this laboratory during the years 1922 and 1923. These experiments were done in cooperation with Dr. F. N. Blau and Dr. John D. Rogers. A large number of extracts prepared from ovaries or *corpora lutea* of pigs and cows were tested especially in regard to their effect upon the sexual organs of the guinea pig. Control extracts of other organs and different solvents have been administered for testing the specificity of the results obtained. The extracts were prepared by Dr. Blau, while the writer made the physiological tests. A careful examination of the vaginal smears of the treated animals was made. A number of animals have also been killed at different stages and their reproductive organs examined in sections. The guinea pigs used as test animals consisted of a group of normal and a group of ovariectomized females. The extracts were given in three different ways—by mouth, by subcutaneous injections and by intracardiac injections.

The results obtained gave indications of different types of reactions, which may be classified in three different groups:

The first group consists only of one set of extracts of lipoid nature prepared from the *corpus luteum*. Such extracts, when given by injections to normal animals, produce certain effects which seem to me entirely characteristic of an active *corpus luteum*. No effect is produced by mouth administration. Under the effect of the injections the large follicles begin to regress in the ovary, the process of ovulation stops and the oestrous or menstrual phenomena are suppressed. An active *corpus luteum* produces the same effects; it prevents the growth of the follicles and suppresses the oestrous or menstrual activities. This reaction seems to be specific and is evidently due to an hormone secreted by the *corpus luteum*.

A second group of reactions is produced by the administration of follicular and cystic fluid extracts and also of ovarian

residue extracts. The effect of these extracts is, as pointed out recently by Allen and Doisy,² to induce congestive and hypertrophic reactions in the genital tract, similar to those produced normally during the oestrous activities. This hormone, though entirely different in action from the first one, is contained also in lipoid extracts and is not effective by mouth.

A third group of reactions is produced by some water-soluble fractions of alcoholic extracts of whole ovaries, and of *corpora lutea*, when given by injections and to some extent by mouth. These extracts stimulate the functions of the sexual organs. The cycle shows a well-regulated periodicity, and a tendency to be somewhat shortened. The ovary functions properly with a slightly increased activity. This stimulating effect is of a different nature from the one produced by the second hormone. This affects more the ovary and stimulates its functions, without producing some of the hypertrophic oestrous reactions in the uterus and the vagina, which characterize the second group. The factor responsible for these effects cannot be confused with the previous ones as it is contained in watery soluble fractions from alcoholic extracts, while the first and second are obtainable only in oil soluble fractions.

The recognition of these three different types of reactions is very important in the final analysis of the ovarian functions. The more active secretions of an hormone of one or the other type may explain why at a certain time the ovary or the uterus reacts in one or the other way. The complicated changes occurring in the sexual organs in a well-regulated cyclic form could not be explained as due to the effect of only one hormone factor. More extensive experimental work which is now under way indicates the possibility of isolating and purifying these ovarian hormones, especially those giving the first and third type reactions. The second type has been greatly advanced by the very important and energetic work of Allen and Doisy.

² *J. Am. Med. Assn.*, 1923, lxxxi, 819-821. *J. Biol. Chem.*, 1924, lxi, 711-727.

56 (2579)

Antigenic relationships of the nucleo-proteins from the gram-positive cocci.

By R. C. LANCEFIELD. (Introduced by Homer F. Swift).

[*From the Hospital of the Rockefeller Institute for Medical Research and the Department of Bacteriology, Columbia University, New York City.*]

Avery and Heidelberger^{1, 2, 3} have shown that the nucleo-proteins obtained from the various types of *Pneumococcus* are species specific rather than specific for type, while the so-called "soluble substance", carbohydrate in nature, is strictly type specific.

We have isolated, from several strains of non-hemolytic streptococci, similar substances which in their chemical and biological behavior show striking similarities to the "specific soluble" substance of pneumococci; they are probably similar to the "residue antigens" of Zinsser,⁴ and his co-workers; and to the soluble precipitating substances of Hitchcock.⁵ Precipitin reactions with the specific soluble substances from green streptococci are highly specific and show close agreement with agglutination reactions; further work on these substances is in progress.

Nucleo-proteins, on the other hand, seem to be serologically similar to each other. Anti-sera, prepared by immunizing four groups of rabbits with the nucleo-protein of four different strains of green streptococci, precipitated the nucleo-protein from five strains of green streptococci, from at least two strains of hemolytic streptococci and from the three type strains of pneumococci as well as from one strain of pneumococcus Group IV.* An immune serum usually precipitated its homologous nucleo-protein a little more strongly than one prepared from a heterologous strain. In the case of the green streptococci the identity of the nucleo-

¹ *J. Exp. Med.*, 1923, xxxviii, 73.

² *J. Exp. Med.*, 1923, xxxviii, 81.

³ *J. Exp. Med.*, 1924, xl, 301.

⁴ *J. Exp. Med.*, 1923, xxxvii, 275.

⁵ *J. Exp. Med.*, 1924, xl, 445, and 575.

* The pneumococcus nucleo-proteins were very kindly supplied by Dr. O. T. Avery.

protein has been confirmed by means of complement fixation reactions. It has been possible to adsorb all of the nucleo-protein precipitating antibodies from an anti nucleo-protein serum by means of nucleo proteins of both homologous and heterologous strains of green streptococci and also by means of one prepared from a hemolytic streptococcus. The results indicate, therefore, that hemolytic and non-hemolytic streptococci and pneumococci all contain a nucleo-protein with common antigenic properties.

Work is also in progress on staphylococcus nucleo-protein. The determination of its relationship to the other nucleo-proteins studied awaits the preparation of more potent sera.

57 (2580)

Local passive immunity against anthrax infection.

By C. IONESCU-MIHAESTI and D. COMBIESCU. (Introduced by Frederick P. Gay).

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.*]

Owing to the varying results obtained in our previous work in which we used guinea pigs as the test animal, the following experiment was carried out on rabbits. Anti-anthrax serum from Mulford & Co. was used with normal horse serum as control.

Ten control rabbits were prepared with normal horse serum: 2 intravenously, 4 subcutaneously, and 4 intradermally; each animal receiving 3 cc. Ten other animals were given anti-anthrax serum by the same routes and in the same dosage.

Thirty hours after the serum injection the resistance of each animal was tested by intradermal inoculation. The rabbits prepared by intravenous injection of the serum were inoculated intradermally over the abdomen. The rabbits prepared subcutaneously or intradermally were inoculated intradermally either at the same point or on the opposite side.

Two to four fatal doses of *Bacillus anthracis* were given. Previous titration of this culture showed that 1/5000 of a 24 hr. agar slant was consistently fatal for rabbits weighing from 1500

to 2500 grams. Two control rabbits, having received no previous treatment of serum, were inoculated intradermally with one and two fatal doses respectively.

Of all the animals prepared with normal horse serum only one survived. This rabbit had been prepared intradermally and later received two fatal doses in the same region.

Both of the animals prepared by intravenous injection of anti-anthrax serum died. Of the four animals prepared by subcutaneous injection of the anti-anthrax serum, one survived. This rabbit had received two fatal doses in the same region as the previous subcutaneous injection of serum.

Of the four animals prepared intradermally with anti-anthrax serum, two survived. These rabbits had received two and four fatal doses respectively, in the same region previously prepared. The two prepared intradermally elsewhere, died.

The rabbits that did not survive, together with two control rabbits that received one and two fatal doses respectively, showed considerable gelatinous oedema around the region of injection and died from 36 hours to 4 days.

This experiment shows that anti-anthrax serum will protect rabbits against otherwise fatal infection of *B. anthracis* under certain conditions. In our experiment the specific anti-serum is preventive only when it has been injected in the area that is subsequently infected. To a less extent normal horse serum will protect a limited area against subsequent infection in that area. It is suggested, therefore, that the prevention of intradermal anthrax infection may be accomplished by local stimulation of cells in the infected area, and this protection is enhanced by the specific properties of an anti-serum.

58 (2581)

The effect of light and of darkness on the growth of the Albino rat.

By HENRY LAURENS and J. W. SOOY.

[From the Department of Physiology, Yale University, New Haven, Connecticut.]

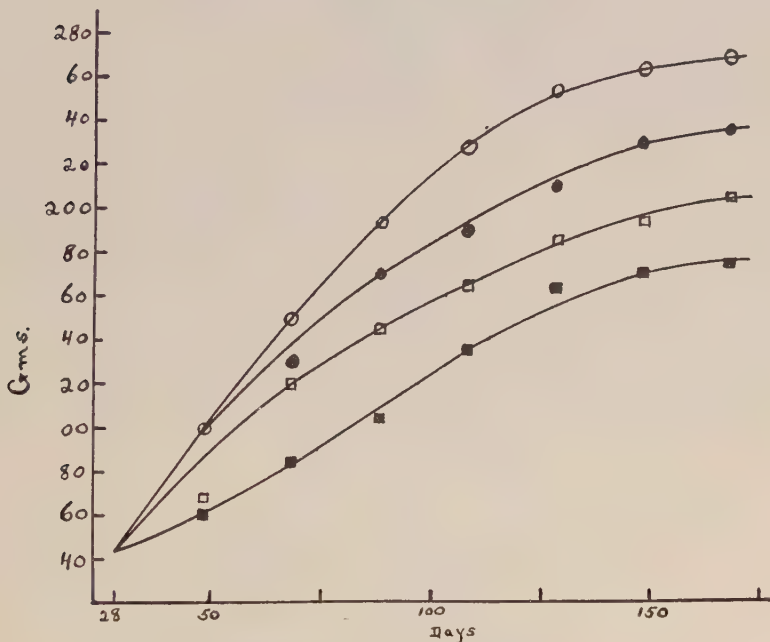
A physiological basis for an explanation of the beneficial influence of visible and ultra violet radiation on pathological conditions may reasonably be expected to be found in a study of the effects of such radiation on "normal" animals. Accordingly we have studied the relative growth of albino rats kept constantly in darkness, of those kept in ordinary room light, of those kept in ordinary room light but given daily exposures to diffuse daylight, and of those similarly given daily exposures to direct sunlight.

The results are of the same nature in all of the series except for seasonal influences such as temperature, ultra violet and visible component of the sun's radiant energy, etc. Our animals have been obtained from time to time from a dealer, the young with the mothers. Our stock diet is Sherman's Diet B. The young are weaned at 28 days of age and distributed in groups of five under the conditions listed above. A series consists of 20 animals. Seven such series, started at different times throughout a year, have so far been observed. A record is kept of the maximum and minimum temperatures, of the relative humidity, as to whether it is bright or cloudy, raining, etc. The dark room, though admitting no daylight, is well ventilated. A 15-watt ruby lamp burning daily for not more than 15 minutes provided the illumination for feeding and weighing.

Only one series will be referred to at the present time, namely a male series E, March 7—July 25, 1924. It differs from the others only in illustrating the most marked evidence of the accelerated growth of the rats given daily exposures to sunlight and diffuse daylight. This is undoubtedly due to the high ultra violet content of the sun's energy at that period of the year.

The curves illustrate the beneficial influence of sunlight and diffuse daylight, perhaps of fresh air, on the growth of a race of rats, which, owing to previous environmental and hereditary influences, is not a superior one as judged from its rate of growth,

on a diet adequate for growth and reproduction. Our colony established, the race is being steadily improved by selection. Observations are being extended to successive generations kept under the four experimental conditions, as well as to the changes induced when individuals are transferred from one situation to another, as for example from sunlight to darkness, as well as to duration of reproductive life, size and number of litters, etc.



DESCRIPTION OF CURVES

Open circles: growth curve of rats given daily two-hour exposures to direct sunlight.

Solid circles: growth curve of rats given daily two-hour exposures to diffuse daylight.

Open squares: growth curve of rats kept in ordinary room light.

Solid squares: growth curve of rats kept in darkness.

59 (2582)

The effect of light and of darkness on blood cell number of
the growing Albino rat.

By HENRY LAURENS and J. W. SOOY.

*[From the Department of Physiology, Yale University, New
Haven, Connecticut.]*

Rats when 28 days old, at which time they were weaned, were placed in groups of five in the dark room, in the stock room, and given daily exposures of about two hours to direct sunlight and to diffuse daylight on the roof as described in the preceding paper. Counts were made of the blood cells at the ages given in the tables under the four conditions indicated. The red counts were made from the first drop of blood exuding after the tip of the tail was severed. Both a direct and an indirect method were used for counting platelets. Details will be given in a later paper. The same pipettes were used throughout for all counts.

Aside from reestablishing the "normal" count at these ages, the stimulating influence of direct sunlight and diffuse daylight, perhaps of fresh air, and the inhibitory influence of darkness on the reds and platelets is obvious from the table. The figures given for the reds represent the averages of counts on five rats, *i. e.*, 30 rats under each of the four environmental conditions, at the ages indicated. The whites and platelets have been studied on a much larger scale.

The whites show a range of counts so wide that the average is of no real significance. The differential shows a decided drop in the lymphocytes of the rats kept in darkness for six months, and a corresponding increase in the polymorphonuclears. The significance of this change is questionable.

	Reds (in hundred thousands)					Platelets (in thousands)			Whites				
	1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	6 mo.		6 mo.				
								Platelet-red cell ratio	Total	Differential			
						Direct method	Indirect method			Polys.	Lymphs	L. monos.	Eosin
Roomlight	22	28	38	45	64	73	762	1 : 10	9,060	30	62	3	5
Sunlight	22	30	39	68	88	110	863	1 : 13	7,560	31	66	2	1
Diffuse daylight	22	27	38	60	82	95	747	1 : 13	8,000	28	65	3	4
Darkness	22	26	30	42	51	60	568	1 : 11	9,720	68	26	4	2

60 (2583)

A method for counting blood platelets in the rat.

By J. W. SOOY and HENRY LAURENS.

[*From the Department of Physiology, Yale University, New Haven, Connecticut.*]

Methods for counting platelets are either direct or indirect. As noted in the preceding paper we have used both. In our hands an indirect method, based on that described by Cramer, Drew and Mottram¹ using the diluting fluid recommended by Rees and Ecker² has yielded the most reliable results. The fluid is 3.8 per cent sodium citrate, to which 0.2 percent formaldehyde and 0.1 percent brilliant cresyl blue are added. It is filtered clear every three days. The same pipettes and counting chambers have been used throughout.

The tip of the tail is shaved, then scrubbed clean and coated with vaseline. With vaselined scissors about 1 cm. of the tail is clipped off, the skin on the stump pushed forward and the bared tip quickly immersed in the solution and a single drop of blood allowed to flow into the fluid. Immediately thereafter, with the red pipette, filled to the 0.5 mark, blood is sucked up from the tail stump to the 1.0 mark and then again with diluting fluid to the 1.01 mark. This gives the sample for the direct red and platelet count.

The solution containing the first drop of blood is mixed thoroughly for a few moments, a drop withdrawn, placed upon a thoroughly cleaned slide, covered with a clean cover glass and sealed with paraffin. After standing, in order that the reds and platelets may settle out, a proportional count is made. Usually three hundred red cells are counted.

By the use of this solution the platelets take on a light blue stain and their structure is more distinctly made out. This, coupled with their examination by means of an oil immersion objective, minimizes the possibility of mistaking other refractile bodies for platelets. We find that in the direct method there is a considerable clumping of platelets as the table in the preceding

¹ Cramer, Drew and Mottram, *Proc. Roy. Soc.*, London, 1922, B xciii, 449.

² Rees and Ecker, *J. Am. Med. Assn.*, 1923, lxxx, 621.

paper shows by comparison with the counts made by the indirect method.

Our thanks are expressed to Dr. A. Bliss Dayton of the Department of Internal Medicine for his assistance and criticism.

61 (2584)

Observations on the theory of tetany.

By IRVINE H. PAGE.

[*From the Research Division of Eli Lilly and Company, Marine Biological Laboratory, Woods Hole, Massachusetts.*]

It has been found that in the sea urchin egg within certain time limits isotonic solutions of sodium phosphate at various $[H^+]$, inhibit cell division only by the limiting effect of the hydrogen ion concentration of the external solution. By direct analysis of the eggs it has been shown that within limits the $PO_4 =$ anion does not penetrate.

Eggs treated with isotonic phosphate over a wide hydrogen ion range, subsequently placed in isotonic electrolytes, and finally placed in sea water to determine their viability, show that (1) KCl has practically no injurious effect, (2) NaCl is most toxic with the peak of minimum toxicity at 7.4-7.8, (3) $CaCl_2$ is toxic with the minimum peak at 7.8, (4) $MgCl_2$ has relatively little toxicity but markedly shifts the peak towards the alkaline side.

$CaCl_2$ and $MgCl_2$ are remarkable in that eggs removed from the ovaries and placed directly into isotonic solutions of these salts even on the most vigorous washing and centrifuging do not cytolysed while the same eggs if allowed to stand in sea water for $\frac{1}{2}$ hour or over, agglutinate and cytolysed immediately. Dr. Heilbrunn¹ has independently confirmed this point. This very extraordinary change is of interest from the point of view of the theory of agglutination and ageing.

Our experiments are directed towards the investigation of the mechanism of tetany. With such simple protoplasmic systems as the sea urchin egg many more environmental factors are under our control than in cells in organoid form. Inasmuch as phos-

¹ Heilbrunn, L. V., Personal Communication.

phates seem intimately concerned in the syndrome of tetany it becomes of interest to find out whether they may cause a pathological unbalancing of the cell—probably of the calcium equilibrium—in such a way that the cell is open to injury by non-specific tetanogenic ions or molecules. The above experiments seem to show that the egg is rendered very susceptible to poisoning, especially by NaCl, when the egg was previously treated with phosphate. This observation suggests Greenwald's¹ theory of tetany—a poisoning by the sodium ion.

California Branch.

University of California, October 18, 1924.

62 (2585)

Some biochemical notes on *Ariolimax californicus*, Cooper.

By D. B. DILL and R. E. SWAIN.

[*From the Department of Chemistry, Stanford University, Calif.*]

Ariolimax californicus is a species of land mollusk common in the vicinity of Stanford University. It is striking because of its yellow color and large size.

The blood of this mollusk is blue when oxidized and the blood ash contains little or no iron but much copper. It is, therefore, concluded that the oxygen carrying protein is hemocyanin. Urea, uric acid, ammonia and creatine + creatinine were found in the blood by qualitative tests. It contained 2.2 percent solids, 0.35 percent ash, and 0.22 percent total nitrogen.

Extracts of the salivary glands, stomach, intestine and liver were examined for the presence of enzymes. The salivary glands yielded a diastatic enzyme which was most active on the acid side of neutrality. Diastase, lactase, maltase and emulsin were found throughout the alimentary tract. Proteolytic enzymes could not be detected by experiments *in vitro*.

¹ Greenwald, I., *J. Biol. Chem.*, 1923, liv, 285.

That the nephridium possesses a true excretory function was indicated by its composition. On the fresh basis, it contained 0.5 percent uric acid (Folin-Denis method) and 0.016 percent of creatine + creatinine (Folin-Benedict method).

The rate of glycogen consumption was determined in liver and muscle while the mollusks were held without food. The glycogen content of the muscle, as determined by Pflüger's method, decreased from the normal of about 30 percent (dry basis) to 19.4 percent after the animals had been held at room temperature without food for fifteen days. In another lot, held in the refrigerator fifteen days, the muscle glycogen dropped to 5.4 percent.

The liver glycogen decreased from the normal of 11 percent to a minimum of about 1 percent after the animals had been held at room temperature for six days. It remained at this level until the experiment was discontinued on the fifteenth day. Essentially the same changes in liver glycogen were noted in those animals held in the refrigerator. It appears from these observations that the liver glycogen of this mollusk is the reserve which is first drawn upon, while the muscle glycogen constitutes a secondary reserve.

63 (2586)

A micromethod for nephelometric estimation of uric acid and purine bases.

By DR. OCT. COQUELET.* (Introduced by K. F. Meyer).

[*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.*]

In an endeavor to develop a microchemical method for the determination of uric acid and purine bases it was found that uric acid was quantitatively precipitated, when in solutions as dilute as 1/10,000 by a modified Salkowski reagent. Graves and Kober, for nephelometric determinations, suspended similar silver purine

* Belgian Fellow of the C. R. B. Educational Foundation at the University of California.

precipitates in egg albumin. This procedure was found unsatisfactory. Efforts to maintain a uniform suspension by reducing the surface tension and augmenting the viscosity were also unsuccessful. In the absence of other salts it was found, however, that a silver-urate-precipitate remained uniformly suspended for one hour, which permitted satisfactory nephelometric determinations.

Technique for Determinations:

(Reagent. 40 cc. of a 5 percent ammoniacal silver nitrate solution is placed in a 250 cc. volumetric flask with 40 cc. of either 5 percent ammonium chloride or 8 percent sodium chloride. Ammonia is then added until the silver chloride is dissolved plus an excess of 10 cc., and the whole made up to volume.)

Uric acid, in a lithium carbonate solution, is delivered in amounts ranging from 0.1 to 2 mg. into centrifuge tubes, to these are added 5 to 10 cc. of the reagent. The resulting precipitate is separated by centrifugalization, the liquid drained off and the inner lip of the tube dried with filter paper. Concentrated HCl is then added drop by drop until the silver urate is entirely in solution. The addition of water to this mixture produces a turbidity which is quantitative for the amount of uric acid present. The dilution must be made in a strictly uniform way. The most satisfactory method is to allow the desired amount of water (15, 20 or 25 cc.) to rush in suddenly from a burette. These solutions are then ready for nephelometric readings. The results can not be calculated by colormetric formulae. The following equation may be used:

$$x = \frac{10 \times St}{R + a(R-10)}$$

where the standard is set at 10, St represents the concentration of the standard, R the reading of the unknown, and a is a factor to be determined for each nephelometer.

Results obtained.	
Expected	Found
0.38 mg.	0.40 mg.
0.54	0.60
1.29	1.295
1.29	1.23

Application: In biological solutions the proteins are best removed at the boiling point by means of colloidal iron and sodium

sulphate. Chlorides and phosphates are determined by a preliminary titration with 0.5 percent silver nitrate, and if present the following procedure is used. The precipitation is made as usual but it is treated with 1 percent HCl instead of the concentrated HCl and held in a water bath for a few minutes. Purine bases and uric acid alone pass into solution and can by this means be separated.

The method will be applied to the determination of purine bases as soon as they can be procured in pure state; however, no doubt exists that the method will be applicable for their determination.

64 (2587)

The production of a hemolytic substance in young liquid cultures of Cl. botulinum.

By P. SCHOENHOLZ. (Introduced by K. F. Meyer).

[*From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.*]

A clear zone of hemolysis is usually noted around each colony of Cl. botulinum when the organism is grown anaerobically on blood agar medium at 35° C. for 3 to 4 days. The blood cells within the hemolyzed area are entirely dissolved. Preliminary experiments to demonstrate the production of a soluble hemolytic substance in 24 hour sheep's serum veal broth cultures were unsuccessful. Subsequent tests indicate that the hemolytic substance is formed during the first stages of growth; it is in all probability destroyed by the 24th hour.

The demonstration of the lytic substance depends on (1) the composition of the culture medium, (2) the age of the culture, and (3) the strain. Plain veal infusion broth is unfavorable for production of the lytic agent. The addition of small amounts of glucose or of 20 percent heated (56° C.—30°) sterile sheep's serum stimulates its formation. The first visible signs of growth in a 1 percent glucose veal broth medium, inoculated with spore forms (3-4 day beef heart cultures heated at 80° C.

for 10 minutes) are usually noted after 14 to 16 hours incubation at 35° C. Hemolysin can be demonstrated in such a culture after 16 to 18 hours growth and usually persists until the 22nd or the 26th hour, depending on the composition of the medium. The lytic substance invariably disappears from the culture by the 38th hour of growth. After the inoculation of young actively growing vegetative forms (12 to 14 hour cultures), the first signs of turbidity usually appear after 4 to 6 hours incubation. About 2 hours later hemolysin can be demonstrated. The peak of hemolysin production is reached after 8 to 10 hours growth; by the twelfth hour the concentration of the lytic agent has either decreased or entirely disappeared. Twenty-four hour cultures are non-hemolytic.

Estimations of the viable organisms made simultaneously with the hemolysin tests reveal that the greatest outpouring of the lytic substance takes place during the logarithmic period of growth. Multiplication *per se* is not the only prerequisite of hemolysin production, since certain vigorously growing cultures may remain entirely inactive to red cells. However, it undoubtedly plays an important part in the accumulation of the substance in the medium.

The determination of the hemolytic activity of a number of Type A and Type B strains has shown that individual variations may exist. Two Type A strains grown in suitable culture mediums invariably failed to form hemolysin. Very young growths (4 to 8 hours) were indifferent to the red blood cells, while older ones darkened but did not dissolve the erythrocytes. However, one of these cultures tested on a blood plate produced a small zone of hemolysis. The failure to demonstrate the lytic agent during the growth of the organism in liquid mediums has as yet not been explained. The data at hand indicate that Type A are less active in hemolysin production than Type B strains.

The hemolysin is very sensitive to the action of heat, light and mechanical agitation. It is readily adsorbed by diatomaceous earth. Exposure to a temperature of 60° C. for 10 minutes destroys it completely. At room temperature the lytic substance gradually becomes weaker; the rate of disappearance depends on the age of the culture which is exposed to the deleterious influences. Centrifugalization at high speed may destroy it; occasionally a clear supernatant dissolves the red blood cells. The detailed results will be published in the near future.

65 (2588)

The hepatic anaphylatoxin. Final evidence of its rôle in canine anaphylaxis.

By W. H. MANWARING, V. M. HOSEPIAN, F. I. O'NEILL and H. B. MOY.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*]

In previous papers,^{1, 2} it was shown that typical anaphylactic contractions of certain smooth muscle structures do not take place on intravenous injection of specific foreign protein into dehepatized anaphylactic dogs. The conclusion was drawn that the characteristic smooth muscle contractions in intact dogs are due to chemical products (hepatic anaphylatoxins), explosively formed or liberated by the anaphylactic liver. We have obtained additional evidence in support of this conclusion by cross-circulation, hepatic transplantation and blood transfusion tests.

As a preliminary to these tests it was shown that primary reactions between specific foreign protein (horse serum) and anaphylactic blood are in themselves insufficient to produce recognizable anaphylactic reactions in normal canine tissues. If a normal dog is exsanguinated as completely as possible and transfused from an anaphylactic donor, the normal dog will show no recognizable anaphylactic phenomena on immediate intravenous injection with specific foreign protein. This finding is in line with the well-known latent period in passive anaphylaxis.

If the isolated hind-quarters of a normal dog are connected by means of paraffined rubber tubes with the general circulation of an anaphylactic dog, typical anaphylactic contractions of the normal urinary bladder and of the normal rectum occur on intravenous injection with specific foreign protein. If the isolated liver of an anaphylactic dog is connected with the general circulation of a normal dog, the normal dog will show a typical anaphylactic fall in arterial blood pressure, typical contractions of the urinary bladder and of the gastro-intestinal tract, and a typical loss of blood coagulability, on intravenous injection with specific

¹ Manwaring, W. H., Hosepian, V. M., Enright, J. R., and Porter, D. F., *Proc. Soc. Exp. Biol. and Med.*, 1924, **xxi**, 536.

² Manwaring, W. H., Enright, J. R., Porter, D. F., and Moy, H. B., *Proc. Soc. Exp. Biol. and Med.*, 1924, **xxii**, 61.

foreign protein. We believe these experiments furnish conclusive evidence of the existence of toxic hepatic products in the general circulation, at least during the initial stage of canine anaphylactic shock.

If blood is drawn from the carotid artery of an anaphylactic dog from two to five minutes after throwing the dog into anaphylactic shock, and this blood is immediately transfused into a partially exsanguinated normal dog, no recognizable anaphylactic phenomena usually occur. This finding is in line with the negative results previously reported by Weil.³ If, however, the foreign protein is injected directly into a mesenteric vein of the anaphylactic dog and shock blood is collected as it escapes from the liver, this blood, transfused into a normal dog, will reproduce all of the characteristic features of canine anaphylaxis. One hundred cc. of this hepatic blood, transfused into a 10 kg. normal dog, will cause a typical fall in arterial blood pressure lasting thirty minutes, typical contractions of the urinary bladder and of the gastro-intestinal tract, and a typical, usually complete loss of blood coagulability. The toxic hepatic products in themselves, therefore, are apparently sufficient to account for all of the observed anaphylactic phenomena.

The shock in normal dogs connected with an anaphylactic liver is less prolonged than the shock in intact anaphylactic controls. The toxic hepatic products, therefore, are presumably not the only factors operative in canine anaphylaxis.

66 (2589)

Changes in glycogen content of the liver in anaphylaxis.

By F. I. O'NEILL, W. H. MANWARING and H. B. MOY.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*]

Changes in the glycogen content of the liver in canine anaphylactic shock were followed by routine histological and chemical methods.

³ Weil, R., *J. Immunol.*, 1917, ii, 399.

The normal canine liver has a fairly constant glycogen content. Stained by Best's carmine method, the parenchyma is usually seen to be fairly well filled with red granules. The granules are usually largest and most numerous in the central portions of the lobules. Quantitative determinations by the official method adopted by the American Agricultural Chemists show a glycogen content varying from 4 percent to 7 percent of the gross liver weight, an average of 5.3 percent in our series.

During typical canine anaphylactic shock (kymograph control), the hepatic glycogen practically disappears. The central half of each lobule often becomes free from stainable granules within three minutes. The whole liver becomes microscopically glycogen-free by the end of fifteen minutes. Less than 0.01 percent glycogen can usually be isolated from the liver at this stage.

No conclusion is as yet drawn as to the mechanism of this glycogen disappearance, nor as to its bearing on fundamental theories of anaphylaxis. Our findings, however, are in line with the initial hyperglycaemia in guinea pig anaphylaxis, recently reported by Zunz and La Barre.¹

67 (2590)

Preliminary note on the effect of a constant magnetic field on morphogenetic processes.

By E. J. LUND.

[*From the Puget Sound Marine Biological Station, and the University of Minnesota.*]

It is generally supposed that a *constant* magnetic field has no effect upon life processes. Observers who have dealt with this question seem on the whole to be in quite general agreement that all attempts up to the present to demonstrate an effect have been complete failures, or else the conditions of the experiment have been too poorly defined to demonstrate an effect. In view of such a situation it is clear that any contrary statement of value must be supported by results obtained under clearly defined experimental conditions.

¹ Zunz, E., and LaBarre, J., *Compt. Rend. Soc. Biol.*, 1924, xci, 121.

Along the bottom of a narrow, long glass trough 1x1.5x15 cm., through which a slow current of fresh sea water was kept flowing, were placed sets of internodes of the hydroid *Obelia*.¹ On opposite sides of one end of the trough were placed the N and S poles of an electromagnet, through which passed a direct electric current kept constant during any one experiment. The internodes were therefore fixed in definite positions along an intensity gradient of magnetic field. The small electric current (2 to 100 milliamperes in different experiments) passing through the coils of the magnet did not produce noticeable temperature change in the magnet; and the stream of running sea water had the same temperature to within 0.1° C. in all parts of the trough.

In brief, the experiments gave a clear proof that growth was entirely inhibited, and inhibition was followed by death in the high field-intensity opposite the poles of the magnet. The degree of inhibition decreased with increase in distance from the poles, until in a position in the outer end of the trough twelve centimeters from the center of the pole faces, no perceptible effect occurred. Thinking that the effect was due to the flow of sea water which generated electric currents in the magnetic field, experiments were performed in which the trough was filled with sea water kept at rest. The effects were the same as in the current of sea water. The effective threshold of field-intensity lies in a range between 6 and 50 Gauss.

Experiments with cut pieces of *Tubularia* stem have yielded similar results. Cell cleavage and development of the *Fucus* egg may be inhibited under the same conditions of experiment. However, in field-intensities of similar magnitude, no inhibitory effects were observed on cell division in *Paramecium*, in the development of the eggs of the fresh water perch, frog, and pond snail, *Planorbis*. In the case of the frog's egg, exposure for seven hours—beginning at the two cell stage—to a constant field of 6150 Gauss had no trace of any effect on development. Results from experiments on the sand dollar (*Echinarachnius*) egg are at present in doubt.

Methods for an accurate quantitative study of the phenomena have been worked out, but limits of space prevent their full presentation here, and the discussion of all the results which have been obtained.

¹ Lund, E. J., *J. Exp. Zool.*, 1924, xxxix, 357.

68 (2591)

The source of energy of the sulphur bacteria.

By L. B. BECKING.

[From the Laboratory for Economic Biology, Stanford University.]

Bacteria which deposit sulphur, either within or outside the cell, are supposed to derive this substance from the oxidation of hydrogen sulphide (Winogradsky,¹ Buder²). This hydrogen sulphide is supposedly the source of energy of these autotrophs.

In an investigation of the external and internal milieu of the sulphur bacteria (to be published elsewhere) the author has investigated mass cultures of the following nine genera:

Thiorhodaceae: Thiospirillum, Chromatium, Rhodochromatium, Amoebobacter. Thiopedia, Thiopolycoccus, Lamprocystis.

Thioleucaceae: Beggiatoa, Thiothrix.

Among other points it was noted that the organisms occurred exclusively in alkaline waters (pH 7.6-8.6) either fresh water, seawater or brine.

As H_2S is a weak acid it will undergo hydrolytic dissociation in aqueous medium, and this dissociation will progress with a decrease in $[H^+]$. We will have:

$$k_1 [H_2S] = [H^+] [HS^-] \quad (1)$$

in which $k_1 = .91 \times 10^{-7}$ at $18^\circ C$.

$$k_2 [HS^-] = [H^+] [S^{2-}] \quad (2)$$

in which k_2 is very small, probably about 10^{-15} . We have also

$$[B^+] + [H^+] = [OH^-] + [HS^-] + 2[S^{2-}] \quad (3)$$

in which B^+ represents total metal ion.

$$k_m = [H^+] [OH^-] \quad (4)$$

From these four equations $[H_2S]$ can be calculated

$$[H_2S] = \frac{([B^+] + [H^+]) [H^+]^2 - k_m [H^+]}{k_1 [H^+] + 2k_1 k_2} \quad (5)$$

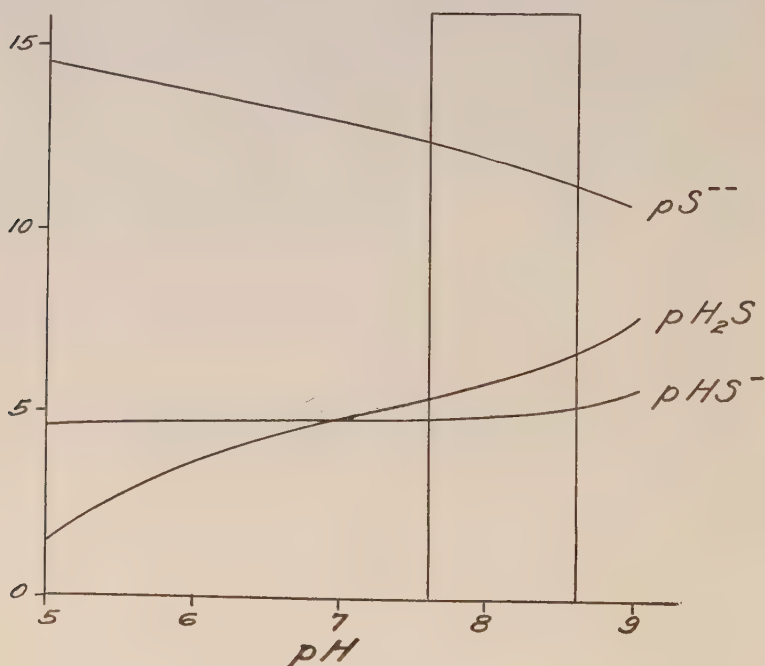
¹ Winogradsky, S., *Bot. Ztg.*, 1887, xlv, 488.

² Buder, J., *Jahrb. Wiss. Bot.*, 1922, vii, 231.

Assuming the metal ions to be present in a low concentration, let us say 10^{-5} , the other ionic concentrations can be plotted

pH	pH ₂ S	pHS ⁻	pS ⁻⁻
5	1.66	4.79	14.70
6	3.92	4.96	13.96
7	4.96	5.00	13.00
8	6.00	5.04	12.04
9	7.66	5.70	11.70

A graphical representation is given in the accompanying diagram.



As can be seen from the graph there is from 50 to 100 times more hydrosulphide ion than undissociated hydrogen sulphide in the natural milieu of the sulphur bacteria. There is, therefore, no *a priori* evidence that H_2S *per se* is the source of energy of the S bacteria. Keil has failed to replace the H_2S by sulphides, in the case of *Beggiatoa*. The author of this paper found .01 percent CaS decidedly beneficial to the growth of *Lamprocystis*. The amount of H_2S in equilibrium with the metal ion is, as we have seen, dependent on the pH. It is apparent from Keil's data that most of the H_2S in his solutions was decidedly in excess of the

metal ion. This gives more evidence that H_2S *per se* is inassimilable, perhaps even toxic to the bacteria.

Arranging the sulphide and hydrosulphide ion and H_2S according to their free energy levels in relation to liquid sulphur and sulphate ion, it will be seen that H_2S aq. has a lower energy

S^{--}		level than the sulphur. It is therefore unfit
	20000 calories	to serve as a source of energy for the sulphur bacteria unless a compensating oxidation of the hydrogen would occur. As the
HS^-		oxygen tension in the black mud (the natural habitat of the bacteria) is extremely low, it
	3000	seems more natural to assume that some
S		other substance, like the HS^- or the S^{--} ion
	6500	will be the source of energy. The low concentration of the latter ion in natural waters,
H_2S aq.		however, points to the importance of the
	170000	HS^- ion as the source of energy. Dehydrogenation of this ion in the absence of oxygen would imply the
SO_4^{--}		presence of a hydrogen acceptor in the same sense as used by Hopkins in his glutathione theory. A mechanism of sulphur formation on the basis of this theory will be developed elsewhere.

69 (2592)

The effect of light on the permeability of lecithin.

By L. B. BECKING and M. I. GREGERSEN.

[*From the Laboratory for Economic Biology, Stanford University.*]

Plant protoplasm changes its permeability in the light (Lepeschkin,¹ Tröndle,² Blackman³). These changes in permeability account for changes in turgor, and inasmuch as turgor-changes precede or accompany the photo-growth reactions in plants, the latter reactions might ultimately be traced to changes in permeability.

After Hansteen-Cranner's⁴ careful ultra-microscopic observations, once more our attention is called to the importance of the lipid component of plant protoplasm. The universal distribution of these lipoids justified our choice of lecithin as a material for permeability studies.

Membranes of lecithin-collodion were prepared in the following way. An ether solution containing 5 percent lecithin and 5 percent collodion, was carefully poured on glass plates, yielding membranes from 10 to 20 μ thick. The manipulations were carried on in the dark.

The dry membranes were mounted in the following way (Fig. 1). Rims were blown on pyrex glass tubes. After grinding the rims and covering them with soft paraffin the membranes were pressed between two pyrex cells, one of which contained a pair of platinum electrodes. The electrode compartment was filled with twice distilled water, the other compartment with .02 M KCl. Both compartments were sealed with cover slides by means of paraffin. The apparatus was mounted in a vertical position (electrode compartment below) in an asbestos box.

The KCl diffusing through the membrane caused a decrease in the resistance between the electrodes, which decrease could be measured by the Wheatstone Bridge and telephone. Fig. 2, Curve I, shows the rate of diffusion through a non illuminated

¹ Lepeschkin, W., *Ber. d. Bot. Ges.*, 1910, xxviii, 28.

² Tröndle, A., *Jahrb. Wiss. Bot.*, 1910, xlviii, 171.

³ Blackman, V. H., *Ann. Bot.*, 1918, xxxii, 69.

⁴ Hansteen-Cranner, B., *Ber. d. Bot. Ges.*, 1919, xxvii, 1.

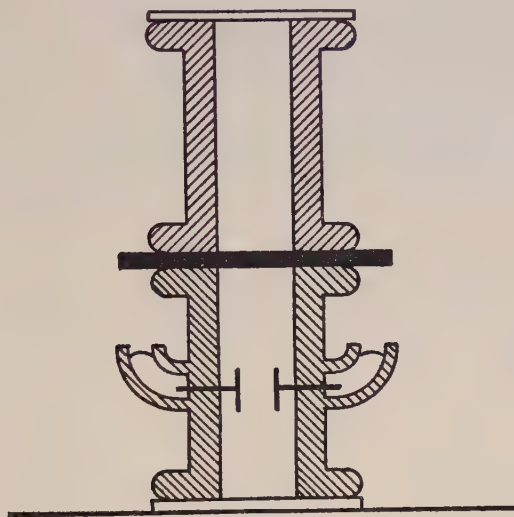


FIG. 1.

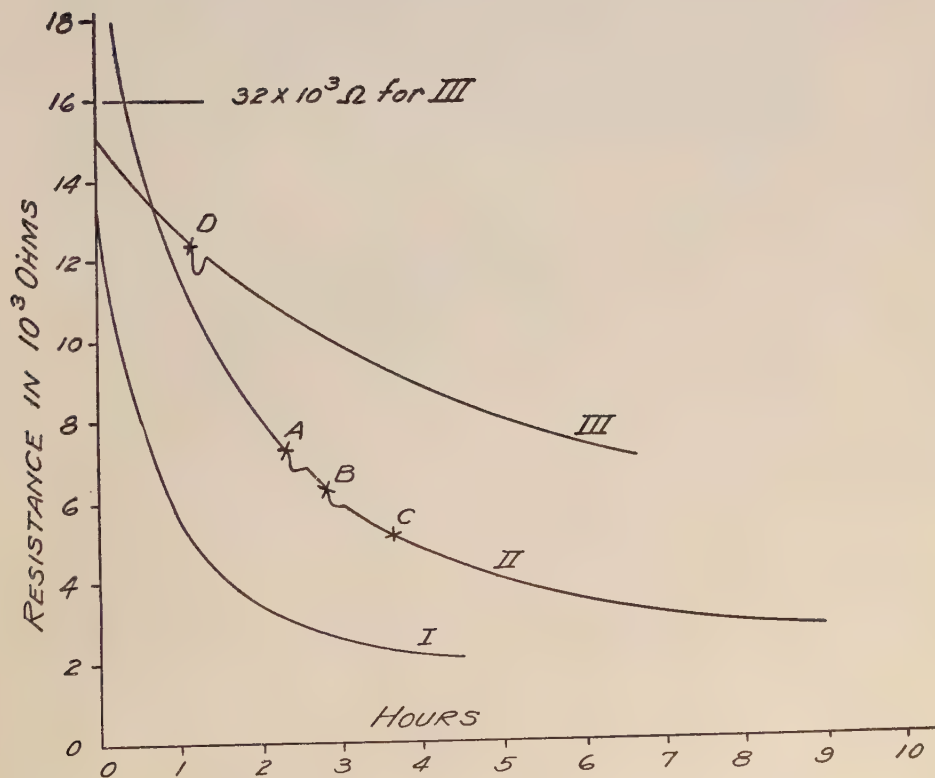


FIG. 2.

membrane. Depending on the nature of the membrane, equilibrium is reached after 9 to 24 hours.

The diffusion curve cannot be represented by a logarithmic line, possibly because of the fact that lecithin dissolves in the surrounding liquid. If x be the conductivity of the liquid, t the observation time in hours, a the initial resistance, we have

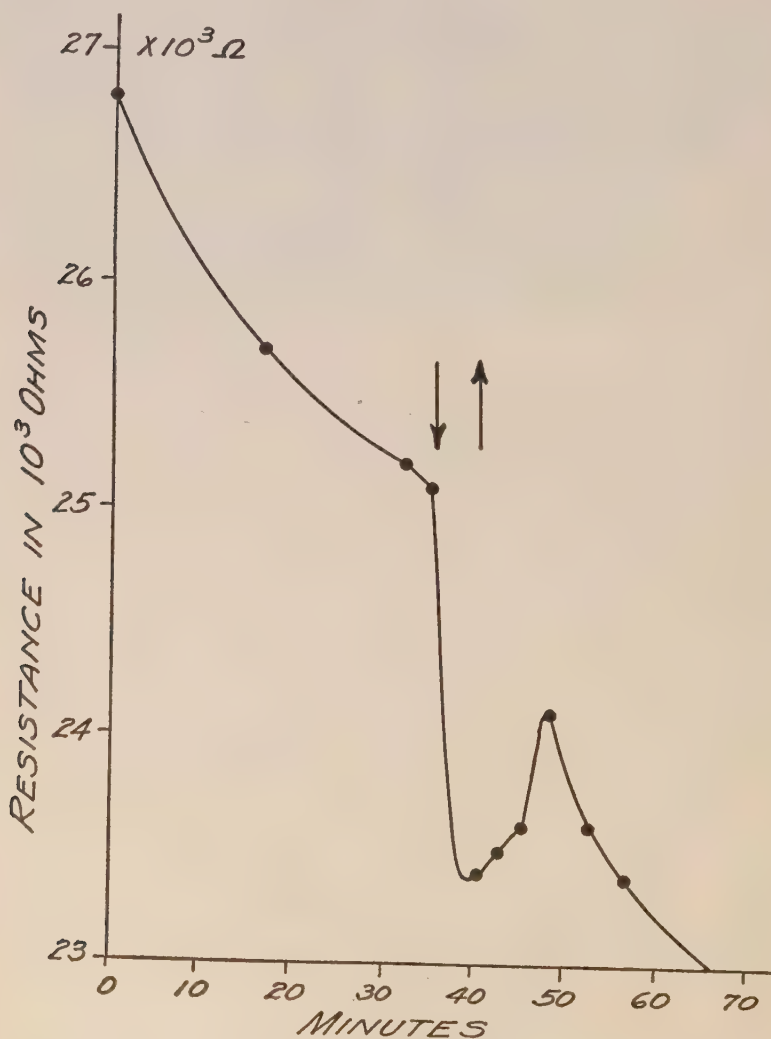


FIG. 3.

been able to represent the diffusion of .02 M KCl through collodion-lecithin by

$$\left(\frac{x}{a} + \frac{1}{c} \log \frac{a}{a-x} \right) = bt, \text{ in which } b, c, \text{ and } d \text{ are constants. In this case}$$

$$t = 9.9 \left(\log \frac{a}{a-x} - .013 x \right) \quad a = 32.5 \times 10^3 \Omega.$$

a-x	t found	t calculated
$15.4 \times 10^3 \Omega$	1 hours	1.10 hours
10.6	2	2.00
7.6	3	3.04
5.7	4	4.03
4.4	5	4.98
3.6	6	5.92
2.9	7	6.81

This general type of curve apparently expresses the resistance-time relation in the majority of cases. Abnormal results, caused by apparent heterogeneity of the membrane, were found when the ether solution contained 15 percent lecithin.

The continuity of the diffusion curve is disturbed by illumination. A water jacketed 300 Watt hydrogen filled bulb was placed at 50 cm. distance from the KCl compartment. Temperature fluctuations during illumination kept within $\pm 1^\circ \text{C}$. Curve II at A, B and C, curve III at D show the effect of five minute illumination of the membrane. There is an increase in conductivity, followed by a more or less pronounced decrease, after which the diffusion assumed its original rate. The effect is more marked on the steep end of the curve, till it becomes imperceptible when the curve flattens out. The effect at III D is represented on a larger scale in Figure 3. The period of illumination is indicated by the arrows. The membranes apparently lose their sensitivity after six to seven days. The experiments, which are still in progress, indicate that lecithin-collodion membranes change their permeability to potassium chloride on illumination.

The lecithin used was one of Dr. P. A. Levene's preparations. We are also indebted to Dr. D. T. Mac Dougal, whose hospitality enabled us to carry out part of the work at the Coastal Laboratory of Carmel, California.

SCIENTIFIC PROCEEDINGS.

70 (2593)

Melanuria in Mental Disease.

By JOHN W. CHURCHMAN, M. D.

[*From the Department of Hygiene, Cornell University Medical College, New York City.*]

In an examination of microscopic sections of the large intestine, removed by Dr. J. W. Draper from about 170 patients suffering from various types of functional neurosis at the New Jersey State Hospital under the direction of Dr. Henry Cotton, marked pigmentation has been noted as a striking feature in many of the specimens. The pigment in these cases contains no iron, and belongs in the group of melanins. It is sometimes present throughout the entire large bowel, but is usually most marked in the caecum, diminishing toward the sigmoid. On section, the pigment is found as a rule to be confined to the mucosal layer, where it is seen as large polyhedral cells with a yellowish brown stippling. It does not stain by the Prussian Blue method. Pigmentation of this type is generally regarded as a sign of intestinal deterioration, and is not infrequently seen in the bowels of those who have suffered from long continued intestinal stasis and intoxication.

Sometimes the epithelial cells themselves are the site of this pigmented deterioration. But more often the yellowish brown polyhedral cells lie in columns in the lympho-reticular tissue, frequently in close proximity to the minute blood vessels. In one instance a pigment cell has been observed lying within the lumen of a small vessel which perforated the *muscularis mucosae* and terminated in the reticular substance of the mucosal layer. These observations suggested the possibility that the pigment in the type of case under consideration might reach the circulation, and should therefore be sought in the urine.

Urinary examinations for the detection of melanuria have been made in 300 patients suffering from various types of mental disease, but otherwise free from disease which might be expected to produce melanuria. The technique used was that employed by Haden and Orr, which was based on the recommendations of Helman.¹ Three reactions in sequence must be demonstrated to prove the presence of melanin: (a) the addition of ferric chloride gives a brown or black precipitate, which (b) dissolves on the addition of sodium carbonate and from which (c) a brown or black amorphous powder is precipitated on the addition of a mineral acid.

In the 300 cases examined, melanin in quantity was five times found in the urine, an observation not previously made in this type of disease. Since a large percentage of patients suffering from mental disease has been proven by pathological studies to present marks of advanced cellular deterioration in their large bowels, since this deterioration is often characterized by a striking degree of melanotic pigmentation, and since the possibility that this pigment enters the circulation has been established by microscopic investigation one explanation of the presence of melanuria in the patients examined which must be seriously considered is that the pigment reached the urine from the bowel. If this explanation proves to be correct melanuria may be found to be an important clinical sign of intestinal deterioration. It is important in this connection to note that Haden and Orr have observed melanuria in intestinal obstruction.

The work here reported is part of an intensive study of the relation of intestinal infection to systemic disease, now in course in the Department of Hygiene at Cornell University Medical School in conjunction with Professor Torrey and Dr. Kahn; and the pathological material was obtained from the Pathological Laboratory of the New Jersey State Hospital.

¹ *Johns Hopkins Hospital Bulletin*, 1924, xxxv, 58.

71 (2594)

The isolation of the *B. histolyticus* from the ileo-caecal region of two human intestines.

By JOHN C. TORREY.

[From the Department of Hygiene, Cornell University Medical College, New York City.]

The *B. histolyticus*, a member of the gram-positive, spore bearing group of anaerobes, is a strongly proteolytic and markedly pathogenic organism, which was first discovered and described by Weinberg and Sequin¹ in the course of their study of war wound infections. They isolated it from eight cases of gas gangrene, and demonstrated its remarkable proteolytic action on living muscular tissue. It was assumed that it found its way into wounds through fecal polluted soil, as was known to be the case as regards *B. tetani*, *B. welchii* and other typically intestinal organisms. This theory received substantiation through the report of Hall² of the presence of this organism in the stool of a normal adult and its isolation from arable soil by Petersen and Hall.³

During the course of an investigation of intestinal bacteriology and absorption of intestinal bacterial products which is being carried on in this laboratory, we have had the opportunity to study the types of bacteria present at various levels of human large intestines removed at colectomy operations. I am indebted to Dr. John W. Draper for this privilege, and to my associate, Dr. John W. Churchman, for obtaining the specimens at the time of operation.

Of ten intestines thus far examined, two have yielded this *B. histolyticus*, and both at the same levels of the intestine. One of these cases was a severe epilepsy in a boy (H) which had been present in an aggravated form since early childhood; the other (C) was a case of chronic and obstinate constipation with a condition of marked stasis in the caecum and colon.

¹ Weinberg and Sequin, *La Gangrene Gazeuse*, Masson et Cie, Paris, 1917.

² Hall, Ivan C., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **xxi**, 198.

³ Petersen, E., and Hall, I. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **xx**, 502.

Cultural studies of the material at various segments in the intestine, which were separated by ligatures at the time of operation, showed in both instances the presence of *B. histolyticus* in predominant numbers, as far as the spore bearing anaerobes were concerned, in the lower ileum and caecum. In the lower ileum it far outnumbered other spore bearers, and in the caecum seemed to occur in numbers exceeding 1000 per mg. of the material. Lower down in the large intestine it apparently did not thrive so well; in the colon there was evidence of its presence but it was considerably outnumbered by *B. welchii* and other spore bearing anaerobes, and in the sigmoid region no evidence of its presence was observed. The material from the latter region, however, consisted only of scrapings from the intestinal wall.

The strains of *B. histolyticus* isolated from these cases were submitted to careful cultural and pathogenic tests, and were found entirely typical. They exhibited a strongly proteolytic action on milk casein, gelatin and cooked minced meat medium, producing in the latter the characteristic white balls of tyrosin crystals. In all tests these strains showed this typical peculiarity, namely, proteolysis without foul odors and with little or no gas production. None of the carbohydrates tested, glucose, lactose, saccharose, salicin, xylose, or insulin, were fermented.

The type of lesion which follows the injection of *B. histolyticus* into muscular tissue of experimental animals is unique and in itself of great differential value, with a fairly virulent strain 0.5 cc., of a 24 hour broth culture inoculated into a thigh muscle of a guinea pig will cause in 24 to 48 hours extensive digestion of the muscular tissue with marked hemorrhage but without gas formation. Comparative virulence tests with these intestinal strains, isolated from the ileums of these two cases, disclosed a marked difference in virulence. Inoculations, of graded amounts of 24-hour casein digest broth cultures, were made into the adductor muscles of large guinea pigs. It was found that 0.1 cc. of the H strain was sufficient to cause typical lesions, whereas 1.0 cc. of the C strain was required to produce a similar effect.

The fact that, except for the finding by Hall of this organism in a stool specimen, there has been no previous report of its isolation from the human intestine, would seem to indicate that it is very rarely present in this locality. M. C. Kahn⁴ has recently

⁴ Kahn, M. C., *J. of Infect. Diseases*, 1924, xxv, 423.

completed in this laboratory a study of the spore bearing anaerobes present in stool specimens from 72 individuals, 60 of whom exhibited symptoms more or less definitely related to the intestine, but did not find the *B. histolyticus* in any instance. I believe, however, that it occurs in the human intestine more commonly than these results would seem to indicate, and that failure to find it in stool specimens may be due to overgrowth with other organisms of the same group, such as *B. sporogenes* or *B. welchii*.

The question of the possible harmful effect of this toxin-producing organism, when vegetating in the intestine, will be discussed in subsequent communications.

72 (2595)

Changes in virulence and growth characteristics of bacterium leipsepticum following alterations in oxygen tension.

By LESLIE T. WEBSTER.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

The micro-organism of the pasteurilla group associated with rabbit snuffles and its complicating pneumonias, septicemias, etc., is known as *Bacterium leipsepticum*. Quite recently, De Kruif noted that a mutation of freshly isolated strains of this microbe, which took place in extract broth cultures, was favored by high concentrations of peptone, and was inhibited by undiluted serum or beef infusion. The recently isolated virulent strain which he designated as Type D was found by him to grow diffusely in serum and plain broth, to form rather opaque, fluorescing colonies on serum agar, and to have an acid agglutination zone of pH 3 to 3.5; while the mutant, which he called Type G, exhibited granular growth in fluid media, appeared as translucent, non-fluorescing colonies on serum agar, showed an acid agglutination optimum between 3.5 and 5, and was of low virulence.

This apparent physical change in the organism, together with the general distinguishing characteristics of each type, has been

noted by us in many of our strains derived from both normal and infected rabbits. So far the pure Type D strains have shown a similar high degree of virulence for rabbits and mice, and all pure G strains have shown a similar low degree of pathogenicity for these same animals. Present studies on growth activities described in this paper, however, necessitate a different interpretation of the D to G transformation and the accompanying drop in virulence.

If numbers of Type G organisms varying from one to one billion are inoculated into tubes containing 5 cc. of extract broth pH 7.4, growth will occur in every tube. If Type D microbes are inoculated into extract broth tubes in a similar manner, multiplication will not occur in tubes which have received a seeding of less than about 50,000. However, if to the extract broth a small quantity of rabbit's blood is added, Type D, inoculated in the smallest numbers, will multiply. Analyzing this phenomenon more carefully by hourly counts after inoculation, it was seen that Type G in plain broth multiplied immediately without lag, and reached a maximum of one billion per cc. in 10 to 12 hours; and that microbes D in plain broth not only failed to grow but died unless at least 50,000 were seeded per tube. On the other hand, the addition of small amounts of rabbit's blood initiated growth of Type D when a very small number of microbes (1 to 10) was seeded and furnished conditions suitable for a logarithmic multiplication of the bacilli reaching a maximum of one billion per cc. in 8 to 10 hours.

When tubes of plain broth inoculated with Type D were examined after an incubation period of 48 hours or more, a granular film was seen at the surface of the liquid which settled rapidly when agitated. This material was found to consist of Type G bacteria. This phenomenon did not occur when blood was added to the broth. It seemed possible, therefore, that the D to G transformation might be associated in some way with oxygen tension; and to test this supposition a series of broth tubes which had been kept in boiling water for 30 minutes and then sealed with vaseline was inoculated with varying numbers of Type D microbes. It was noted that abundant growth was present in every tube, including those which had received the smallest possible inoculum, and that subsequently no D to G transformation occurred. Evidently, then, limiting the oxygen supply to extract broth tubes, or adding rabbit blood to this medium under aero-

bic conditions, facilitates growth and inhibits the D to G transformation.

This observation suggested the possibility that an oxidation reduction process, possibly such a peroxidase-peroxide system as McLeod and Avery have independently described, might be concerned in this type mutation with accompanying loss of virulence. The activity of the blood in inducing optimum growth of Type D microbes and inhibiting the D to G transformation was tested and found to function in a dilution of 1/40,000 of a cc., a quantity just sufficient to give a positive benzidine test. Washed and autoclaved red blood corpuscles in a similar high dilution to the limit of the benzidine reaction behaved in a similar manner. This evidence indicated so strongly that blood was behaving as a peroxidase, that a synthetic compound was tried, $\text{Fe}(\text{OH}_2)(\text{NC})_5\text{Na}_3$, with strong catalytic properties, suggested by Doctor Baudisch and kindly put at our disposal by him. This preparation of iron reacted in dilutions of 0.002 mg. per cc., giving a positive benzidine reaction, furnishing optimum growth conditions for Type D, and inhibiting the appearance of G forms.

Peroxidase is present in the concentrated suspensions of Type D cells; in similar suspensions of Microbe G, the test is approximately four times as strong. So far we have not been able to demonstrate peroxide in the cultures.

Bacterium lepi-septicum may be considered, then, as an organism so delicately adjusted in its oxygen requirements that it fails to multiply freely or to maintain certain characteristics associated with its virulence unless available oxygen is mechanically limited, or the oxygen effect is minimized by the presence of peroxidase.

73 (2596)

The extraction of alkaloids from blood.

By ROBERT A. HATCHER.

[From the Department of Pharmacology, Cornell University Medical College, New York City.]

When certain alkaloidal salts are added to defibrinated blood of the cat the bases can be extracted by shaking the blood with chloroform. Strychnin has been recovered in this way from a

million parts of blood. The method is applicable to codein, heroin, and quinin, and almost certainly to nearly all the common vegetable alkaloids. Morphin is extracted less readily than most of the alkaloids.

About 20 per cent of an intravenous dose of codein was recovered from blood drawn immediately after the injection. Quinin was recovered from blood drawn at once after the intravenous injection in the cat, but very small amounts were present in the defibrinated blood.

Urine and bile may be extracted in the same way with slight modifications.

The defibrinated blood of the cat yields less than a milligramme (as a rule) of chloroform-soluble matter.

74 (2597)

The relation of adrenalin to the action of insulin upon the blood sugar content.

By E. F. MUELLER, M. J. LEWI, and C. N. MYERS.

[From the Department of Dermatology and Syphilology, College of Physicians and Surgeons, Columbia University, New York City.]

During the course of experimental work on the effect of insulin upon the blood sugar content in rabbits, the question arose as to whether insulin and adrenalin were antagonists.

It is well known that insulin acts by diminishing the blood sugar, while adrenalin increases it. It was, therefore, thought highly probable that the administration of both drugs would result in a neutralization of these effects so that the blood sugar would remain practically normal.

A study of the literature gave support to the assumption. Magenti and Biagotti conclude that adrenalin, when given simultaneously with insulin, acts by strongly disturbing the usual insulin effect. These tests were repeated only for the reason that in the first papers on insulin by Banting and Best, and especially in a recent article by McLeod and Orr, special attention was called to the individual differences in rabbits, for sugar test, after the administration of insulin.

A series of rabbits was tested primarily for the separate effects of insulin and adrenalin.

Insulin was injected in quantities of 0.5 units per 1 kg. body weight. This amount was chosen because our former work, on the differing effects of insulin in different body tissues, was done with like quantities. These quantities seemed sufficient to lower the blood sugar content of normal rabbits markedly, without the danger of an overdosage. Thus convulsions and other by-effects, possibly without determinable manifestation, could be avoided. This seemed necessary because other tests, published recently, have shown that every struggle of the animals is followed by an increase in the blood sugar content, thus disturbing the reading of the exact results of the final findings.

The results of these tests are given in a table, the columns of which represent the values of the blood sugar content in intervals of two hours after the administration of insulin. For an easy comparison the numbers are given in percentage, assuming the original value of the blood sugar in every animal as 0.0.

TABLE I.

Rabbit	Insulin alone subcutaneously		
	2	4	6
58	—63.5	—18.7	4.9
76	—42.5	— 5.5	7.8
78	—62.6	—13.7	— 5.8
79	—40.8	—37.7	—23.8
80	—65.7	— 0.0	0.8
82	—22.8	0.8	5.5
84	— 1.6	— 0.8	1.6
85	—43.0	—15.6	— 8.2
86	—31.6	— 6.7	— 9.6

In the second table the same animals were injected with adrenalin. 0.1 cc. of a solution of adrenalin, 1:1000 was used in each animal, because previous tests developed that this amount of adrenalin would increase the blood sugar, while as much as 0.5 units of insulin would diminish it. Thus, by using the indicated amount of adrenalin combined with 0.5 units of insulin in the first series, a neutralizing result could be expected.

The results of adrenalin in the same rabbits are tabulated as follows:

TABLE II.

Rabbit	Adrenalin alone subcutaneously		
	2	4	6
58	62.3	4.9	— 4.1
76	64.0	26.0	14.0
78	48.8	16.8	0.8
79	30.2	12.2	— 0.6
80	20.2	— 5.6	— 6.5
82	123.0	16.1	—11.5

The columns of Table II demonstrate the blood sugar findings after adrenalin, using the same two-hour intervals as in the insulin tests.

In a third table the results will be found to indicate the changes in the blood sugar content when both adrenalin and insulin were administered:

TABLE III.

Rabbit	Insulin and adrenalin, subcutaneously and simultaneously at different sites		
	2	4	6
58	—33.1	—24.8	5.0
78	—26.8	—41.3	42.3
79	—42.6	—32.5	—25.0
84	13.8	—41.3	28.3
85	26.5	6.0	2.6
76	—45.6	11.9	16.5

The results are not absolutely uniform; but four of the animals unexpectedly showed a decrease in the blood sugar content. In some the four-hour test was surprisingly lower than in the same animal when injected with insulin only.

These findings were more marked when both agents were not given simultaneously, but the insulin injection postponed until 20 minutes after a subcutaneous administration of adrenalin. The results of these findings, tabulated in Table IV, demonstrate unusually low figures in some animals, even in the first and in the second tests.

TABLE IV.

Rabbit	Adrenalin 20 minutes before insulin, at different sites, subcutaneously		
	2	4	6
78	—68.5	—50.0	13.9
80	—40.8	—48.0	— 7.2
82	—19.1	— 6.7	— 0.0
86	—17.1	0.8	— 8.9

An explanation of these facts has hitherto not been furnished. It must be assumed from our experimental experience that the final effect of the injection of insulin and of adrenalin, separately administered, namely the change in the blood sugar content, is not produced by a like mechanism.

If, for instance, insulin would have a direct influence on the liver or on another organ that on its part would cause the blood sugar decrease, adrenalin probably does not act in the same way, merely producing a contrary effect on the organ's function. If so, the action of insulin and adrenalin must eliminate each other like the neutralization of a positive and a corresponding negative power.

In accordance with the findings described, it may perhaps be concluded that adrenalin effects the action of the insulin by blocking its way from the site of administration to the organ which in turn acts upon the blood sugar changes. This assumption would explain the fact, for instance, that in some tests the effect of insulin is postponed and becomes effective after four hours, while usually the principal effect upon the blood sugar takes place two hours after administration.

Neither can the findings shown in Table IV be explained, especially those in which the insulin action is markedly enlarged. Perhaps the stimulation of the sympathetic part of the involuntary nervous system plays a part also in these findings. As there is no definite proof of it, it must be conceded that the reasons are unknown.

Lack of knowledge or of a satisfactory explanation do not affect the importance of a fact. In these interesting biologic processes, represented by the action of insulin and adrenalin in the healthy body, we have had results which must be accepted as positive findings, the cause and significance of which still remain to be established.

The observations have been discussed because they do not agree with the expected results. They show the following conclusions:

1. Adrenalin and insulin, administered to the same animal, do not eliminate their mutual actions.
2. In some instances, adrenalin injections, made simultaneously with the administration of insulin, increase the action of insulin by lowering the blood sugar content more than usual.
3. In some instances, the action of insulin is only slightly

diminished in the first tests (2 hours after administration), while the findings after four hours manifest an increase in the insulin action, usually observed at that period.

4. It has been demonstrated that (in more than 70 percent of the cases examined) the insulin effect is generally not eliminated by adrenalin, but is sometimes enlarged.

5. The reasons for these findings have hitherto not been discovered. They may be based upon an action of the involuntary nervous system.

75 (2598)

Diluting lipid antigen with a constant dropping syphon.

By WILLIAM N. BERG.

[*From the Berg Biological Laboratory, Brooklyn, N. Y.*]

In order slowly and uniformly to dilute an alcoholic solution of tissue lipoids as required in such serologic methods as that of Meinicke,¹ an apparatus was desired that could easily and quickly be set to deliver water dropwise at a definite rate. Numerous trials by Kohler² indicated that the lipid extract should be diluted in such fashion that seven volumes of distilled water are dropped into one volume of lipid extract in twenty-eight minutes. A stable suspension of fine lipid particles is formed, which obviously should be prepared as often as desired under identical conditions. The syphon herein described is simpler than the floating syphon used by Dreyer and Ward.³

It delivers 49 cc. in exactly 28 minutes, but it can easily be set to deliver other amounts. From the figure the construction is almost self explanatory. The syphon itself is made by bending a piece of heavy wall soft glass tubing twice at right angles in the same plane. For convenience it is held in a rubber stopper perforated horizontally. Two capillary buret tips were attached to

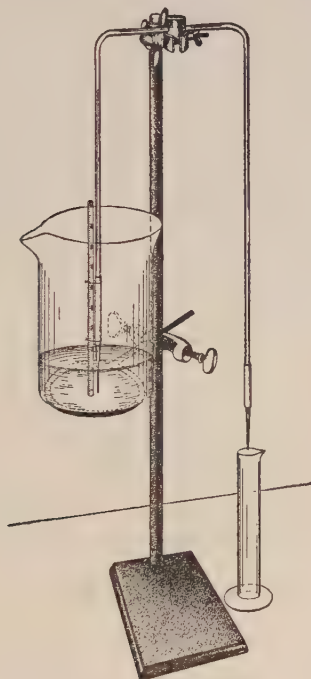
¹ Meinicke, E., *Berliner klinische Wochenschr.*, 1918, Jahrg. lv, pp. 83-96.

² Kohler, E., *Ztschr. f. Infektionskrankheiten d. Haustiere*, 1921, xxi, 288-314.

³ Dreyer, G., and Ward, H. K., *Lancet*, 1921, cc, 956-961.

the limbs of the syphon with rubber tubing, glass ends in contact, so that the water flows through glass only. A broken thermometer stem was cut and attached to the short limb so that its lower end was a fraction of a millimeter below the capillary tip. By using detachable capillary tips, the limbs of the syphon may be cut if desired.

Assuming that the worker is to dilute 7 cc. of a lipoid extract with 49 cc. of distilled water, dropwise, in exactly 28 minutes, it is desired to set or calibrate the syphon so that dilutions may be made as desired without loss of time. The following steps were found useful:



1. Construct the apparatus approximately of the dimensions shown in the figure and given in the table below. The difference in total lengths of the limbs should be near 64 mm.

2. A 1.1 liter griffin form beaker, outer diameter 110 mm., was a convenient water container. Of course any container may be used, but the same one should be used throughout. The beaker is raised or the syphon lowered until the capillary tip and scale just touch bottom.

3. Pour distilled water into the beaker until the depth indicated on the scale is 20 mm.

4. Start the syphon by suction, attaching a rubber tube to the longer limb for this purpose. As soon as the syphon is working, detach the rubber tube, receive the drops in a cylinder and note the time.

5. Note the time required to deliver the desired volume.

If delivery is too rapid, the longer limb may be cut a few millimeters; if too slow, the initial depth of water in the beaker may be increased a few millimeters. After a few trials, quickly and easily made, the exact conditions can be determined. Following are some typical results obtained without any unusual precaution:

Syphon tube, outer diameter, 4.4-4.5 mm., bore, 1.2 mm. Glass beaker, 1.1 liter, outer diameter of base, 110 mm.

Total height of long limb	Total height of short limb	Difference	Initial depth of water	Volume dropped	Time	
					min.	sec.
mm.	mm.	mm.	mm.	cc.		
432	368	64	10	49	31	45
"	"	"	"	"	31	25
"	"	"	20	"	27	12
"	"	"	"	"	27	20

76 (2599)

The effects of varying internal and external pH of *Valonia* upon penetration of arsenic.

By MATILDA MOLDENHAUER BROOKS.

[From the Division of Pharmacology, Hygienic Laboratory, Washington, D. C.]

A study was made of the penetration into *Valonia macrophysa* of trivalent and pentavalent inorganic arsenic by changing both the internal pH of *Valonia* and the external pH of the surrounding solution. The internal pH was decreased by subjecting the cells to NaHCO_3 solution, and increased by treatment with NH_4Cl solution. The external pH was varied from pH 5.0 to 9.0 by adding arsenic adjusted to the desired pH (by adding

NaOH or HCl) before the addition of sea water, and kept constant at that pH by the addition of minimal amounts of HCl. The pH determinations were made with indicators. The cell wall, protoplasm and sap were separately analyzed for arsenic. The method of handling and testing the cells was the same as that used in a previous paper.¹

It was found:

1. That the concentration of arsenic in the sap was less than that in the protoplasm. With trivalent arsenic, the difference is less than with pentavalent arsenic.

2. The minimum amount of arsenic penetrates into the sap and protoplasm, when the external solution is approximately neutral.

3. When free CO_2 is allowed to accumulate in the plant, and the plants are then placed in pentavalent arsenic solutions of various H ion concentrations, more arsenic is found in both sap and protoplasm than in normal plants placed in similar arsenic solutions. When trivalent arsenic is used instead, the concentration of arsenic in the sap is increased, while that in the protoplasm is decreased.

4. When free NH_3 is allowed to accumulate in the plants, and the plants are then placed in pentavalent arsenic solutions of various H ion concentrations, less arsenic is found in the sap and protoplasm than in normal plants placed in similar arsenic solutions. When trivalent arsenic is used instead, more arsenic is found in the sap and protoplasm than in normal plants placed in similar solutions.

5. The pH of the external solution, as well as that of the inside of the plant, affects the rate of penetration of pentavalent and trivalent arsenic. When either or both the external or internal pH values are low, more pentavalent and less trivalent arsenic are found in the protoplasm and in the sap; the opposite is true when the external solution and the interior of the cell are alkaline.

6. There is no difference in the amount of As found in the cell wall under varying conditions; apparently the wall does not affect the rate of penetration of As into the protoplasm and the sap.

7. Differences in the rate of penetration of arsenic as influenced by changes in external pH cannot be explained by attri-

¹ Brooks, M. M., *Public Health Reports*, 1923, xxxviii, 2951.

buting them to dissociation of the acids and subsequent effect on the arsenic in the external solution. These differences in the rate of penetration seem to be due to effects on the protoplasm initiated by changes in both the internal pH of the cell and the pH of the bathing solution.

Details of this paper will be published shortly in the U. S. Public Health Reports.

77 (2600)

Suppurative otitis of the albino rat.

By HOWARD A. McCORDOCK (Fellow National Research Council) and
CHARLES C. CONGDON (Introduced by B. Roman).

*[From the Laboratory of the Buffalo General Hospital,
Buffalo, N. Y.]*

It has been observed in colonies of albino rats that certain individuals become affected with a condition characterized chiefly by the following phenomena: The animal persistently holds its head tilted to one side; instead of running in a straight line it does so in a curve in the direction of the deviation of the head; and when suspended by the tail the body spins rapidly around in the same direction. It was also subsequently discovered that deafness on one or both sides accompanies these symptoms. According to private communications this condition has been observed in various breeding stations in this country, and Greenman and Duhring¹ make mention of this condition under the heading of Middle Ear Disease.

The colony under our observation consists of four strains obtained from different sources which will probably be traced to a common strain. The disease has been studied for the past twelve months, and out of 2,700 animals, twenty-three became affected at various periods. Of these, three died, two apparently from the disease, and one as a result of pneumonia. The other

¹ Greenman and Duhring, *Breeding and Care of the Albino Rat*, Wistar Institute of Anat. and Biol., Philadelphia, 1923, 102.

twenty did not seem very sick, and outside of the symptoms mentioned behaved like normal rats. Nor did the disease entirely prevent reproduction or raising of the young.

Autopsy of such animals invariably revealed a purulent condition of the ear of the affected side, associated with purulent rhinitis, and nothing remarkable in the rest of the organs. Microscopical examination of the affected ear showed the middle ear filled with pus, and osteomyelitis of the auditory bulla (mastoiditis). The inflammatory process could be traced from the middle ear into various structures of the internal ear.

The inflammatory process is characterized by the presence of a dense leucocytic exudate in the lumen of the middle ear and its mucosa, with marked destruction of the latter, and replacement by granulation tissue. There is also an osteitis of the bony ear with extensive bone absorption and excessive new bone formation involving even the base of the skull. Thus, places could be found in many sections of the series where the process from the middle ear was continued into the bony and membranous parts of the internal ear, whereby not only the semicircular canals and the vestibular structures were filled with exudate or granulation tissue, but also the cochlea. Diffuse inflammatory infiltration of both leucocytes and small cells was found in the neighboring soft parts, and in several places in and about the vestibular and cochlear nerves, and in the spiral and vestibular ganglia.

Evidently the peculiar symptoms of this disease represent a disturbance in equilibration based upon a labyrinthitis following an infectious middle ear disease, which in turn is related to the affection of the nose. The inflammation could be traced from the nose through the Eustachian tube into the middle ear.

Thus the process in the middle ear apparently precedes the onset of the above symptoms. It therefore became important to determine the incidence of middle ear disease in the colony. For this purpose a number of animals were killed (mostly discards) and the middle ears examined. It was then found that out of 152 rats, 79 (about 50 percent) had pus in one or both ears. Many of these rats also showed deafness on the affected side.

In contrast to the above, another group of 42 animals were examined in their prime, and of these 9 (about 22 percent) showed pus in one ear only.

It was therefore evident that we were dealing in the colony with an infectious disease starting probably in the nose, leading to suppurative middle ear disease, which occasionally continued into the internal ear, and which gave rise to symptoms of disequilibrium and deafness.

Pathological changes similar to those mentioned above are described in three rats with similar symptoms, by Droogleever Fortuyn.² He utilized the degenerative changes produced in the eighth nerve and its ganglia by the otitis, for the purpose of studying the tracts of that nerve.

Daniels and Armstrong³ have recently found suppurative middle ear disease associated with nasal sinusitis in rats fed on diets deficient in fat soluble Vitamin A. They have demonstrated middle ear disease in six out of sixteen rats, and conclude that the vitamin deficiency plays an important rôle in bringing about the infection. In experiments with deficient diets we found that the percentage of animals infected on a diet deficient in minerals is even greater than in animals on a diet deficient in Vitamin A.

Exciting Cause.—From the bacteriological, serological, and experimental work so far accomplished, it appears that the exciting agent of both the nasal and aural affections is a small gram-negative, somewhat pleomorphic, motile, non-spore-bearing bacillus. In its smallest form it appears quite as short but not quite as slender as the *B. influenzae*. Although initial cultures of this micro-organism grow slowly, later generations grow readily on ordinary culture media under aerobic conditions. Further work with regard to its identity and its immunological characters is in progress. Serum of diseased rats occasionally agglutinates strains of the bacillus in low dilutions.

² Droogleever Fortuyn, Ae. B., *Psychiatrische en Neurologische Bladen*, Amsterdam, 1918, xxii, 211.

³ Daniels, A. L., and Armstrong, M. E., *J. Am. Med. Assn.*, 1923, lxxxi, 828.

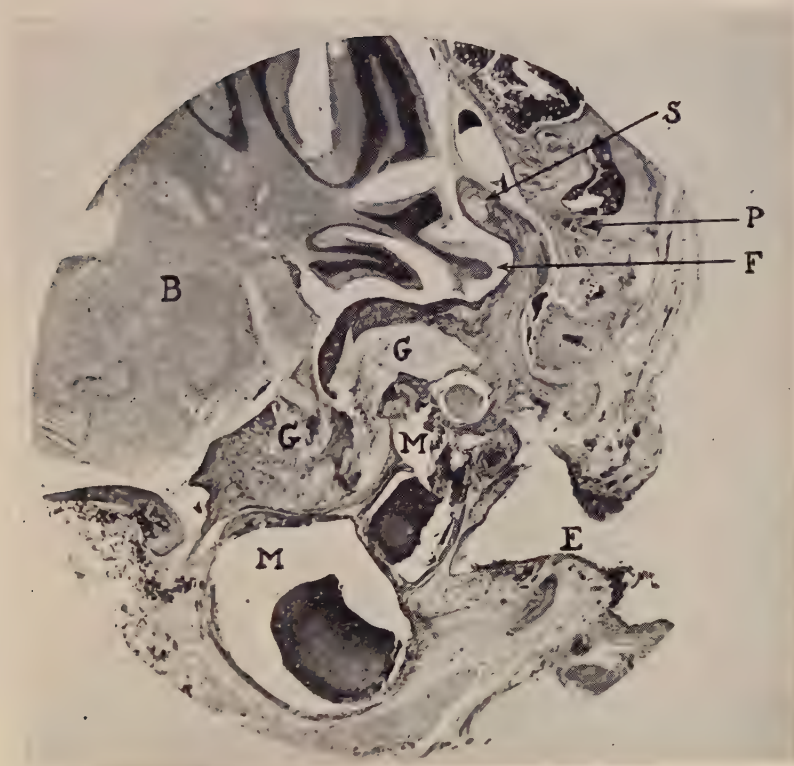


FIG. 1.

Frontal section of head at the auditory organ.

- E.—External auditory meatus.
- M.—Middle ear containing pus.
- G.—Granulation tissue replacing internal ear.
- S.—Acute exudate and granulation tissue in semicircular canal.
- F.—Flocculus of cerebellum.
- P.—Productive osteitis.
- B.—Brain.



FIG. II.

Section as Fig. 1 (higher power).

M.—Middle ear showing pus.

G.—Granulation tissue breaking through bone from middle to internal ear.

S.—Saccule of internal ear.

78 (2601)

A precipitin reaction in epidemic poliomyelitis.

By EDWARD C. ROSENOW.

[From the Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.]

For several years I have used the precipitin reaction, together with agglutination tests, in the identification of various streptococci which culturally and morphologically are very similar. Observing the almost constant presence of a relatively mild infection in the nasopharynx of patients suffering from epidemic poliomyelitis, it occurred to me that a precipitin reaction might be obtained by simply layering the cleared salt solution extract of the nasopharyngeal washings or swabbings over the hyperimmune poliomyelitis streptococcus serum used in the treatment of poliomyelitis.

The first opportunity to test this possibility presented itself between September 20 and October 5, 1924, in four typical cases of acute poliomyelitis which occurred near Rochester, Minnesota. The reaction was positive in all with the poliomyelitis serum, but negative with normal horse serum and various immune serums used as controls. Through the splendid cooperation of the Health Department and the medical profession of Detroit, Michigan, I have been able to apply this test in a large number of cases of epidemic poliomyelitis and poliomyelitis contacts. Moreover, many control tests have been made on persons suffering from miscellaneous diseases and on normal persons, both within and without the epidemic zones. The accompanying table illustrates the results obtained.

In a number of instances in which the nasopharyngeal extract has given strongly positive results with the hyperimmune horse serum, positive results have also been obtained with the serums of patients convalescing from poliomyelitis.

Results of precipitin experiments in epidemic poliomyelitis.

Groups	Number of persons	Positive			Negative
		Mildly	Strongly	Total	
Poliomyelitis	Proved cases	25	42	67	15
	Abortive cases	3	4	7	1
	Total	28	46	74 (82%)	16*
Normal contacts		11	18	29 (76%)	9
Miscellaneous diseases	In Rochester	1	0	1 (4.8%)	20
	In Detroit	7	6	13 (65%)	7
Scarlet fever	In Detroit	4	1	5 (24%)	16
	In Chicago	2	0	2 (9.5%)	19
Normal persons	In epidemic zone, Detroit	34	19	53 (59%)	37
	Outside epidemic zone, Rochester and Chicago	5	0	5 (5.5%)	85

*The 16 tests which were negative were all made 15 days or more after onset of symptoms.

79 (2602)

The relation between the body-weight and age of the human fetus.

By RICHARD E. SCAMMON and LEROY A. CALKINS.

[From the Department of Anatomy, University of Minnesota, Minneapolis, Minnesota.]

In earlier papers in this publication^{1 2} we have suggested the following empirical formulæ for the relation between body-length and age, and between body-length and body-weight:

$$(1) \quad T = 2.3 + \frac{2.5L}{28} + \frac{L^2}{284}$$

$$(2) \quad W_d = (0.26L)^{3.108} + 4.6, \text{ or } L = 3.846 \sqrt[3.108]{W_d - 4.6}.$$

$$(3) \quad W_1 = (0.26L)^{3.108} + 223.9, \text{ or } L = 3.846 \sqrt[3.108]{W_1 - 223.9}.$$

In these formulæ, T is the menstrual age in fetal or lunar months, L is the total or crown-heel length of the body in centimeters, W_d is the weight of the dead body in grams, and W_1 is the body-weight in the living in grams.

To these may be added another expression for the relation between body-length and age, namely:

$$(4) \quad T = 2.134 + 0.1L + 0.0011L^2.$$

The ages computed by this formula for 5 cm. stages of body-length from 5 to 50 cm. inclusive, show an average absolute deviation of 0.063 fetal months, or 1.77 days from the corresponding observed values of Mall³ as determined by interpolation. The average percentage deviation is 1.28. While these deviations are somewhat larger than those of formula (1) above, the present expression is more useful, for it holds good from 2.5 fetal months to birth, whereas (1) holds good only above three fetal months.

A formula for the relation between age and body-weight may be obtained from (4) by substituting weight in terms of length as given in (2). This is:

$$(5) \quad T = 2.134 + 0.3846 \sqrt[3.108]{W_d - 4.6} + 0.01627 \sqrt[1.554]{W_d - 4.6}.$$

¹ Scammon and Calkins, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 353.

² Scammon and Calkins, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 549.

³ Mall, Determination of the age of human embryos, and fetuses; Keibel and Mall, *Human Embryology*, 1910, i, 199.

This expression is too clumsy for practical use and we have therefore computed the values from it and approximated simpler expressions for them as follows:

$$(6) \quad T = 3.0 + 4.049 \sqrt{W_d} - 0.012, \text{ or}$$

$$(7) \quad W_d = 0.561 - 0.336 T + 0.061 T^2.$$

W_d , in this instance, is the dead weight in kilograms. The weights for each lunar month from 2.5 months to birth, as determined by these expressions, are given in Table 1, and are shown graphically in Figure 1.

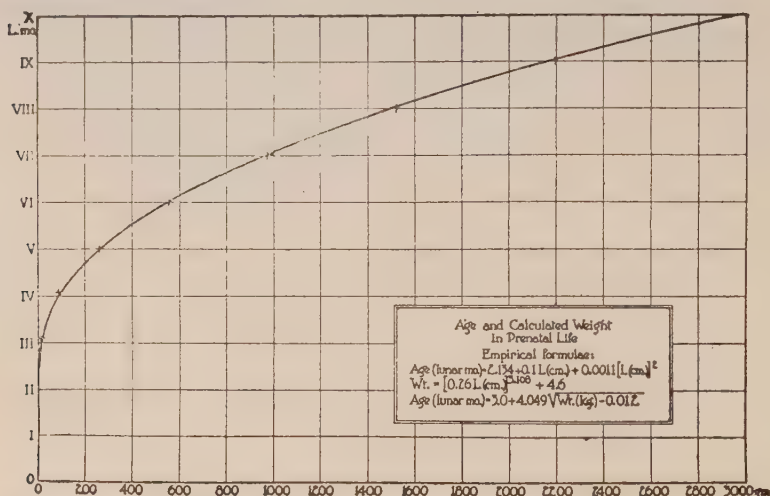


Figure 1. Graph of the growth of the human fetus in weight. Abscissæ body-weight; ordinates, age. The crosses represent values determined by formulæ (2), (4) and (5). The solid line is drawn to formula (6).

It is also desirable to have some expression which will enable us to estimate the age of the fetus from both the body-weight and the body-length. We find that the values obtained in the preceding equations may be approximated by the empirical formula:

$$(8) \quad T = 2.046 + 0.12 L + 0.8 W_d - 0.057 W_d^2.$$

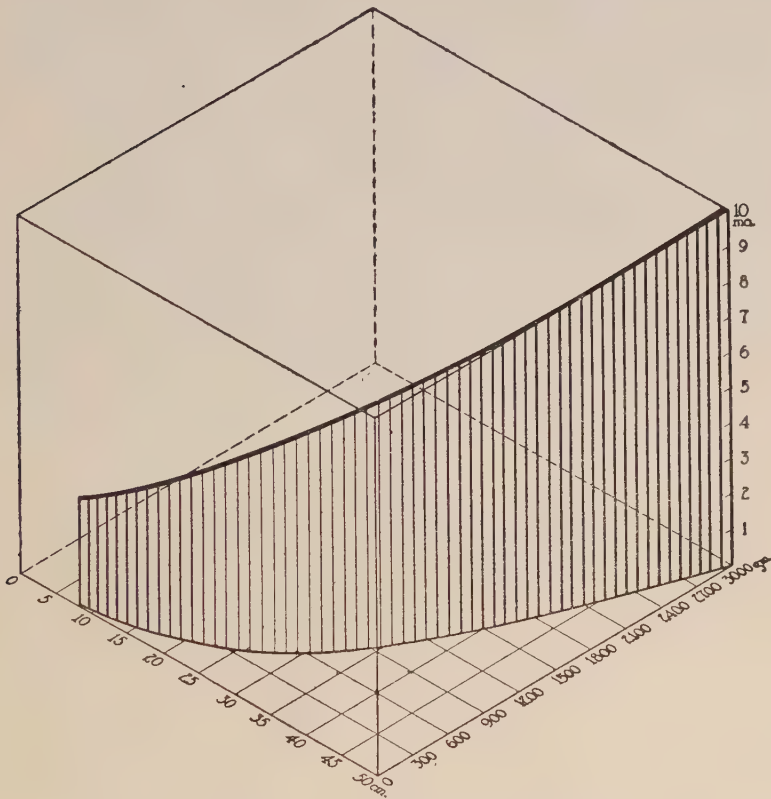
Much simpler forms may also be used such as:

$$(9) \quad T = 2.5 + W_d + 0.1 L, \text{ and}$$

$$(10) \quad T = 2.04 + 0.7 W_d + 0.12 L.$$

While these do not fit the values obtained by other types of formulæ as well as does (8), they are probably close enough approximations for practical use. In all of these formulæ W is

the weight in kilograms. Table 2 gives the values obtained with formulæ (8) and (10) as compared with those of (4). Figure 2 is an isometric projection of the curve of formula (8) showing the relation between the three variables, weight, length, and age, in the fetal period.



Projection of a curve illustrating the relation
between *Body-Length*, *Body-Weight* and *Age* in prenatal life
Computed from the empirical formula:
 $\text{Age (lunar mo.)} = 0.12 \text{ L (cm.)} + 0.8 \text{ W (kg.)} - [0.057 \text{ W (kg.)}]^2 + 2.046$
or, approximately, $\text{Age (lunar mo.)} = 0.1 \text{ L (cm.)} + \text{W (kg.)} + 2.5$

Figure 2. Isometric projection of curve representing the relation between height, weight, and age in the fetal period.

TABLE I.
Age and Calculated Body-weight in the Fetal Period.

Age (fetal months)	Calculated length (cm.)	Calculated dead weight (gm.)		Deviation of results of (7) from (5)
		Exact formula (5)	Approximate formula (7)	
2.5	3.53	3.53	3.63	+ 0.10
3.0	7.99	14.29	12.00	— 2.29
4.0	15.89	86.79	73.00	—13.79
5.0	22.91	260.87	256.00	— 4.87
6.0	29.24	551.61	561.00	+ 9.94
7.0	35.12	971.37	988.00	+16.63
8.0	40.58	1519.09	1537.00	+17.19
9.0	45.70	2196.15	2208.00	+11.85
10.0	50.53	2998.83	3001.00	+ 2.17

Sum 78.83
Mean 8.76

TABLE II.
Calculated age according to length and length-weight formulae.

Body-length (cm.)	Dead body- weight cal- culated from body-length	Calculated age (fetal months)			Deviations of results obtained from "b" and "c" from those obtained from "a"			
		(a) Age calcu- lated from body-length (4)	(b) Age calculated from body- length and body-weight by exact formula (8)	(c) Age calculated from body- length and body-weight by approximate formula (10)	"b"		"c"	
					Absolute (mos.)	Relative (per cent)	Absolute (mos.)	Relative (per cent)
5	6.86	2.66	2.65	2.64	-0.01	-0.34	-0.02	-0.56
10	24.08	3.24	3.27	3.26	+0.03	+0.74	+0.02	+0.52
15	73.31	3.85	3.90	3.89	+0.05	+1.40	+0.04	+1.06
20	172.61	4.57	4.58	4.56	+0.01	+0.26	-0.01	-0.20
25	340.75	5.32	5.31	5.28	-0.01	-0.19	-0.04	-0.77
30	597.03	6.12	6.12	6.06	0.00	-0.05	-0.06	-1.01
35	961.14	6.98	6.96	6.91	-0.02	-0.26	-0.07	-0.96
40	1453.17	7.89	7.89	7.86	0.00	-0.01	-0.03	-0.42
45	2093.52	8.86	8.87	8.91	+0.01	+0.11	+0.05	+0.51
50	2902.85	9.88	9.89	10.07	+0.02	+0.81	+0.19	+1.94
				Sum.....	0.15	4.17	0.53	7.95
				Mean.....	0.015	0.417	0.053	0.795

80 (2603)

Observations on the metabolism of the corallines.

By LAURENCE IRVING and L. B. BECKING.

[From the Hopkins Marine Station and the Laboratory of
Physiology of Stanford University, Cal.]

The results of this investigation suggest a clearer view of the problem of lime deposits and the conditions of organic deposition. The coralline studies are widely distributed and convenient for experiment because of their activity. Although no direct record of their internal changes can be suitably followed, we can determine the changes in the external milieu—changes which will complement those occurring within the organism. In the external milieu, sea water, the carbonic acid-carbonate system, especially with Ca and Mg, is of particular biological significance.

No observations have yet been so distributed as to give complete or even consistent ideas on the whole process of carbonate deposition. Deposition may occur (1) by the accumulation of carbonates incorporated inside of organisms, or (2) by precipitation from the surrounding water by the photosynthetic removal of CO_2 . Removal of single ions from sea water is impossible, and deposits within the tissues appear principally as CaCO_3 . Diffusion of lime into the alga would be facilitated (1) by its precipitation within the organism and (2) by the use of carbonic acid in photosynthesis. Each process would reduce the concentration of these ions within the plant. Diffusion must essentially be, at some point, a factor limiting this process of metabolism.

Titration of sea water to an end point of pH 4.0 with methyl orange gives the excess base, or concentration of weak acid anions, called X-base.

The average (normality) of the sea water was $(25.68 \pm .92) \times 10^{-4}$ N. That of carbonate + bicarbonate, which constitute about half of the X-base, was .0013 N.

Calcium in this sea water was 0.0114 M. If the X-base change of corallines results in an equivalent calcium removal, the amount would be 0.00065 M. As a smaller quantity of magnesium is also removed and its concentration is 0.0507 M, the percent

change would be very small. The X-base changes may be very easily followed; while such calcium and magnesium changes approach the limits of accurate determination.

Corallines were selected as material partly because of their geologic interest. Count Solms¹ finds that the alga must have the power to decompose lime at the growing points. Berthold² claims to have observed that there is little lime deposited in shaded habitats. Both observations seem to indicate that the lime deposition is dependent on external as well as on internal factors.

The large surface of *Corallina*, as compared with the other corallines, and its hardness made us use this form for the experiments. As already shown by Meigen, *Corallina* contains calcite and magnesite. Oltmans³ cited an analysis of Högbom, who found in a *Lithothamnion* from the Bermudas 82.4 percent calcite and 12.4 percent magnesite. About 11 percent $MgCO_3$ was found in a sample of the *Corallina* used in our experiments. Clarke's data⁴ give several other similar analyses. Only 37.8 percent of the organism is water.

Filtered water showed a reduction in the X-base, but the difference was so small that unfiltered sea water was used. The corallines themselves, with their large surface, carry an amount of Epizoa which may interfere with the accuracy of the experiment. Especially the calcareous tubes of a worm, *Serpula*, may cover a large portion of the algal surface, but the material selected was almost free from contamination.

Weighed amounts of the alga were placed in Pyrex flasks. A battery of six flasks was used, through which was bubbled a continuous stream of clean outside air. The air was previously led through sea water to prevent evaporation in the flasks. By this procedure the pH remained constant throughout the experiment for 50 hours or more. Series in which the flasks lost weight were discarded. The upper series of three flasks was illuminated by a 75-watt Mazda lamp, 50 centimeters distant. The lower series was kept in darkness.

Total excess base was determined by titration with .01N HCl against methyl orange. The end point selected was pH 4.0, by

¹ Solms-Laubach, *Monogr. Zool. Station Naples*, 1881, iv, Leipsic.

² Berthold, *Jahrb. f. Wiss. Bot.*, 1882, iii, 569.

³ Oltmanns, *Morph. u. Biol. der Algen*, 1923, iii, 7.

⁴ Clarke, *U. S. Geol. Survey Bull.*, 1920, 695.

comparison with 0.05M KH phthalate. The reduction of X-base and the pH is recorded in the accompanying table.

If the total available excess base for coralline metabolism be assumed to be .0013N (total carbonate), we may duplicate the smoothed tabulated curve by mathematical expressions, "av. b." is base available for coralline use, judging from the amount ordinarily used under such conditions.

Series	Date	Water in gm.	Alga in gm.	Time in hours	X-base light	X-base dark	X-base control	pH
I	16	200	24.5	50	14.90 15.80		25.55	8.55
II	18	200	25	20	14.95 15.10	17.86 17.88	25.65	8.55
V	26	200	5	42.50 66.50 95.59	13.85 13.70 15.98	17.45 16.48 19.34	26.58	8.55
VII	5	200	5	1.15 2.10 3.05 5.45 7.30	24.74 22.51 21.80 19.95 19.70		25.20	8.50
X	20	250	5	8 22.25 23	19 13.50 14.75	22.30 18.90 19.65	25.70	8.55
XII	25	200	5	45 48	15.10 14.63		25.64 18.52	8.50

t (h)	av. b. light	av. b. if $\log \frac{13}{13-x} = 2.9 \times 10^{-5}t$	av. b. dark	av. b. if $x = 1.5 t$
2.5	4.2	3.80	2.	2.37
5.0	6.3	5.50	3.2	3.36
10.	8.6	8.40	4.7	4.75
15.	10.3	10.30	6.0	5.82
20.	11.3	11.32	6.9	6.72
25.	12.0	12.00	7.6	7.50
35.	12.6	12.66	8.7	8.86

On account of our ignorance of the processes involved we can only suggest this empirical analysis. The light reaction is closely expressed by this mono-molecular reaction equation; one substance is acted upon, and the velocity of the reaction is proportional to the amount of that substance present. The dark reac-

tion curve can be duplicated by E. Schütz's law⁵, the intrinsic meaning of which is the following⁶:

⁵ Schütz, *Ztschr. Physiol. Chem.*, 1885, ix, 577.

⁶ Euler, *Chemie der Enzyme*, 1920, Vol. I, 124, 2d ed.

If $x = t$ or $x^2 = a^2t$, differentiation will give

$$\frac{dx}{dt} = \frac{a^2}{2x}$$

In this case the velocity of the reaction will be inversely proportional to the amount of decomposed substance.

	X-base used	2 Ca++	2 Ca++ + X-base used
Sea water	0.0000	0.0228	0.0228
8h light	0.00067		
23h light	0.00109	0.0216	0.0227
8h dark	0.00034	0.0227	0.0230
23h dark	0.00061	0.0224	0.0230

The reduction in carbonate is approximately equivalent to the Ca⁺⁺ removal.

In contrasting the effect of other algæ on X-base each form seems to have its own particular metabolic behavior, which is apparent in this change. Several grams of the coralline *Lithophyllum* were without effect on the X-base. *Ulva* increases the X-base, while the red alga *Gigartina* seems to cause a slight decrease. The effect is enhanced in the light. The brown alga, *Leathesia*, proved to be without effect on the X-base, but changed the pH. A great many metabolic peculiarities of various algæ seem to find their expression in the total excess base. The characterization of the various forms will be found in the table.

Plant 10 gm.	X-base after 24 hours.		pH.	
	Light.	Dark.	Light.	Dark.
Corallina	13.58	18.03	8.55	8.55
Ulva	34.40	30.85	8.65	8.35
Gigartina	24.14	25.20	8.40	8.25
Leathesia	25.25	25.40	8.80	8.60

All plants were collected at the same ecological stratum (*Fucus* zone).

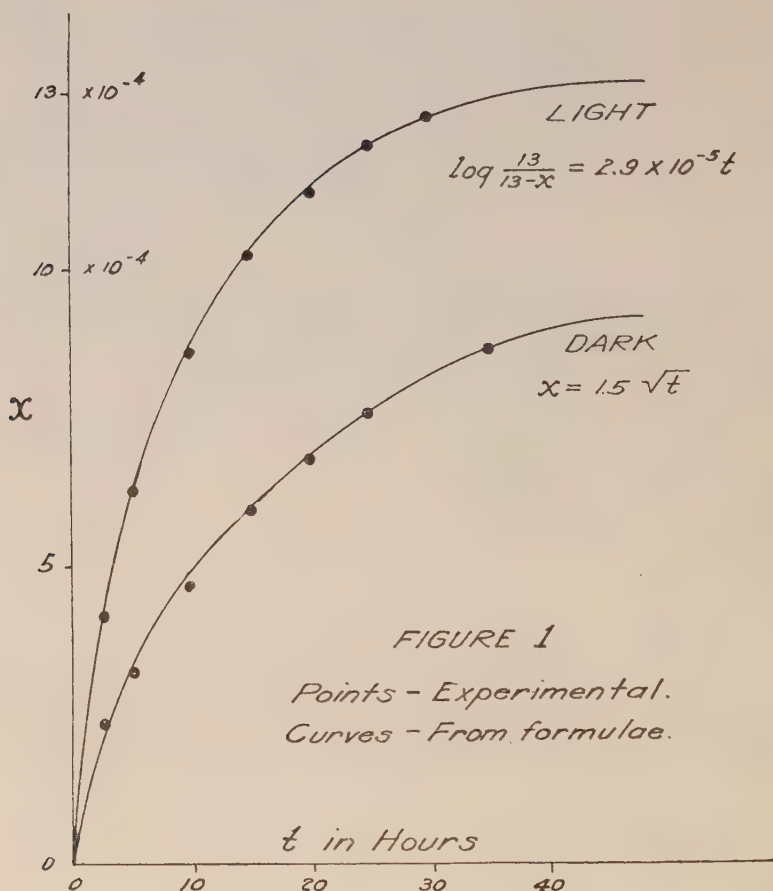


FIGURE 1

Points - Experimental.
Curves - From formulae.

81 (2604)

A method for recording continuous blood pressure.

By MORRIS H. KAHN.

[From the Department of Cardiovascular Diseases, Beth Israel Hospital, New York City.]

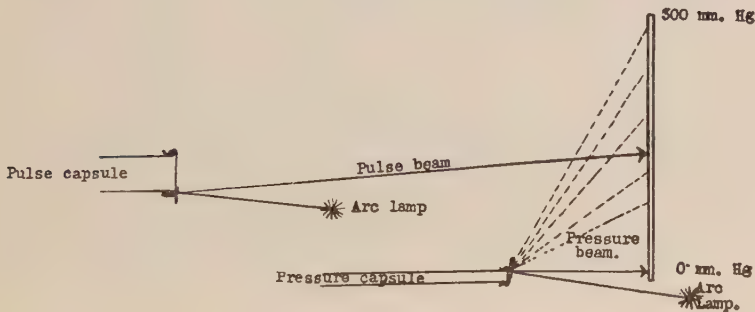
With the usual apparatus used in clinical study, such as the blood pressure manometer or the Erlanger capsule recording on a smoked drum, the pressure changes in the cardio-vascular system

can not be followed accurately. The inertia of the apparatus and the variation in the elasticity and responsiveness of the different instruments give inaccurate or asynchronous data.¹

The application of the Frank capsule method to the study of pressure has been to record pressure curves or pressure gradients by means of an optical system consisting of a mirror on the rubber capsule, reflecting a beam of light.² Its immediate responsiveness and accuracy are extremely efficient.

By a simple means of adaptation, it can be made to record with extreme sensitiveness the amount of blood pressure over a continuous period of time. The rubber dam stretched over the end of a piece of metal or glass tubing is made so tight as to resist pressure up to 300 mm. of mercury. The band of light reflected from the mirror on the capsule is so arranged, directed over the slit of a camera, that its excursion from 0 to 300 mm. of mercury pressure should range from one end to the other end of the slit in the camera. The position of the band of light at any point across the moving film represents a known, previously standardized, amount of pressure (Figure I).

FIG. 1.



This system is connected with a blood pressure cuff and with a mercury manometer. When the blood pressure cuff is inflated and deflated alternately, it records a zig-zag line on the moving film of the camera.

At the same time, another Frank capsule is connected with

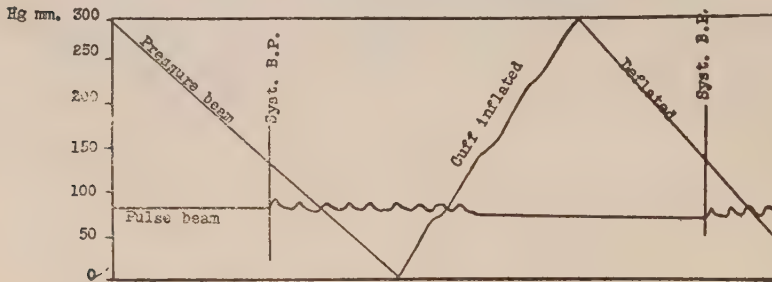
¹ Frank: *Ztschr. f. Biol.*, 1903, xxvi, 445; 1908, xxxii, 309; 1910, xxxv, 429, 545; 1911, xxxvii, 547.

² Wiggers, C. J.: *Arch. Int. Med.*, 1915, xv, 77; *Jour. A. M. A.*, 1915, lxiv, 1485.

the radial or brachial pulse below the cuff by means of a glycerin capsule. The pulse wave appears as another beam of light on the film. It is recorded as a wave when the pressure in the arm cuff is below the systolic point, and as a straight line when the pressure rises above the systolic point. Each time the pulse reappears, the systolic pressure is known from the position of the pressure beam on the film.

A series of these observations is made in sequence, and the point of appearance of the pulse curve can be plotted. This will show the blood pressure variation every few seconds. Diagrammatically, this is represented in Figure II.

FIG. 2.



This apparatus can be utilized to note the changes of blood pressure during and immediately following exercise. The patient's arm must, of course, be at rest as in working a bicycle ergometer. The correctness of the clinical tests based on pulse rate and pressure changes during and following exercise can be analyzed with absolute precision by this method. The controversies in this field are abundant. The physiological basis for the findings has not yet been definitely determined.

82 (2605)

The effect of insulin on the morphological blood picture.

By VICTOR E. LEVINE and J. J. KOLARS.

[From the Department of Biological Chemistry and Nutrition,
School of Medicine, Creighton University, Omaha, Nebraska.]

We have studied the blood picture in rabbits after insulin treatment, and have found that the injection of this hormone produces a rise in the number of erythrocytes per cubic millimeter, and also in the number of leucocytes. The fact that both red and white cells increase simultaneously is strongly indicative of the existence of anhydremia. This condition Drabkin and Edwards¹ have postulated from the increased hemoglobin content following the subcutaneous administration of insulin to dogs. The increase in the red and in the white cells argues more favorably for the existence of a state of anhydremia than the increase in hemoglobin or in the red cells.

Nitzescu and Mangiucca² have recently reported the occurrence under the influence of insulin of a leucopenia followed by leucocytosis. In our experiments, and under the conditions with which we have worked, no leucopenia was ever encountered.

The increase in the white was always greater than in the red count. From this observation we believe that the mild leucocytosis is due partly to the increase in the blood concentration, and partly to the presence of protein and other substances in the insulin preparation. The rise in the number of cell components of the blood was proportional to the fall in blood sugar. The greater the fall in the sugar content of the blood, the greater was the anhydremia. There was apparently no relation between the size of the insulin dose and the degree of anhydremia. As the anhydremia progressed the blood drawn increased in viscosity, and sometimes difficulty was experienced in obtaining for analysis the desired quantity of blood from the ear. This difficulty was probably not due to an increased tendency of the blood

¹ Drabkin, D. L., and Edwards, D. J.: *Am. J. Physiol.*, 1924, lxx, 273.

² Nitzescu, I. I., and Mangiucca, I.: *Compt. rend. soc. biol.*, 1924, xc, 1347.

to clot, for J. la Barre³ found that American insulin does not effect coagulability.

We have made differential counts, and find no appreciable changes in the relative distribution of the various types of white cells.

83 (2606)

Flocculation reactions with hemolytic immune sera.

By K. LANDSTEINER and J. VAN DER SCHEER.

[From the Laboratories of The Rockefeller Institute for Medical Research, New York City.]

As mentioned in the proceedings of the last meeting of this Society, the hemolytic action of the immune sera prepared by injection of alcoholic blood extracts is inhibited by the addition of these same extracts. In analogy to the Forssman heterogenetic antibodies (Sordelli and Pico, Sachs and Guth) these sera were found to give a very distinct flocculation reaction with an emulsion of the extract. The technique is similar to the one used by the authors named with the exception of the omission of cholesterol. The washed sediment of one part blood was extracted with $2\frac{1}{2}$ volumes and again with one volume of alcohol overnight at room temperature, and the mixed alcoholic extracts evaporated to one-half of the original blood volume. To 1 cc. of this fluid 5 cc. of saline were added drop by drop with constant shaking, a homogenous stable emulsion being formed. Mixture of equal parts of the emulsion and diluted ($\frac{1}{2}$ to $\frac{1}{16}$) immune serum were allowed to stand in the incubator for 20 hours.

Similar reactions though with less regularity and intensity were also obtained with anti-horse blood immune sera prepared in the ordinary way, namely, by injections with washed horse blood. Control tests with 22 heterologous hemolytic sera gave a weak reaction in two instances only. The reaction, therefore, has a marked degree of specificity.

Analogous reactions were found with immune sera for other kinds of erythrocytes but their specificity must still be investigated.

³ la Barre, J.: *Compt. rend, soc. biol.*, 1924, xc, 1038.

84 (2607)

The effect of light and of darkness on some urinary and blood constituents in the dog.

By HENRY LAURENS, H. S. MAYERSON and L. GUNTHER.

[From the Department of Physiology, Yale University, New Haven, Conn.]

As a part of the general problem of the influence of light on "normal" animals, a study is being made of changes induced in dogs subjected to longer or shorter successive periods of darkness and of light. At the present time we desire to report a series of results on certain chemical constituents of blood and urine.

Two female dogs, M and G, were brought into the laboratory on November 10, 1923, and placed in large metabolism cages. Dog M was a full grown adult weighing 17.2 Kg., while dog G was about a year old, weighing 12.8 Kg. The room in which the cages were kept was well ventilated and lighted, having a southeastern exposure. Temperature was read on a maximum and minimum thermometer, and registered a maximum variation of 4°C.; while wet and dry bulb readings showed the humidity to be constant.

The diet used was that recommended by Cowgill¹ supplemented by 40 mg. per kilo of Harris yeast vitamine powder made into special tablets containing 50 mg. vitamin extract and 50 mg. inert starch. The diet (see Table I) furnished 80 calories per kilo body weight, and has been shown to be ample for the maintenance of dogs under ordinary laboratory conditions.

The dogs were allowed to become accustomed to the food and surroundings, until analyses of urine and blood indicated nitrogen balance. Catheterization was done daily with due precautions against infection. Usually the urine was analyzed immediately. When this was impossible the sample was covered with toluene and kept in the refrigerator. The cages were cleaned daily to prevent contamination of the urine by hair and feces. When desired for analysis, blood was drawn from the femoral artery under oil, immediately after catheterization.

¹ Cowgill: *Journ. Biol. Chem.*, 1923, lvi, 725.

Standard methods were used in the urine analyses. The Folin-Wu tungstic acid system was used in the blood determinations. Ca and P were done according to Kramer and Tisdall.

On December 15 the room was made light proof. This was done in preference to moving the cages into another dark room in order to maintain the conditions as constant as possible. Feeding and taking of samples was done by the aid of a 15-watt ruby lamp aided by a small flashlight when such proved necessary. Extreme precautions were taken to guard the animals against undue exposure to even this amount of light.

The animals, though agitated for the first few days, as evidenced by incessant barking, gradually quieted down and soon seemed happy, consuming all their food regularly. On January 21 the shades were removed and light again admitted to the room. Once more the animals gave evidence of excitement, but again soon quieted down. Analyses were continued until January 31.

Table II shows the results of the analyses during the preliminary light period, the dark period and the subsequent light period. When the room was darkened the urine and the blood nitrogen increased in both dogs, more markedly in dog M, although dog G showed a proportional rise in blood non-protein nitrogen. In urine nitrogen, the change was latent. No analyses were made between December 18 and January 12, and it is probable that the rise observed on the latter date for dog G took place much earlier. There was no marked change in the percentage value of the various nitrogenous constituents. The phosphates showed no definite change in the urine, while the chlorides did. Blood sugar rose slightly. The acidity of the urine was at a higher level. Both dogs increased in weight. Many of the values decreased and were approaching normal at the end of the dark period.

On the day following the readmittance of light, there was a rise in nitrogen similar to, but proportionately more marked in dog M than that which occurred at the beginning of the dark period, and again of greater extent than in dog G. This rise soon subsided, and the constituents were already approaching their normal levels one week after light was admitted. Although urinary N values are lower than normal in the latter part of the dark period, we should hesitate to say that there was nitrogen

retention because of the small number of determinations carried out.

Other similar series of the same and of much longer duration have been run with comparable results.

TABLE I.

		Cal.	Percent
13.1% N, 80p.c. pure			
Casein	6.1 gm.	19.4	35.06
Sucrose	7.15	28.6	41.1
Butter fat	1.22		
Lard	2.33	32.0	20.4
Bone ash40		3.44
Salt mixture (Karr)20		
		17.40	80.
			100.

Kilo unit contains 0.8 gm. N; 1 gm. contains 4.59 calories.

TABLE II.

Dog M		Urine										Blood									
Date	Wgt.	Vol.	Spec. Grav.	Acidity	Total N	Urea N	Creatinine	Creatinine	Phosphates	Chlorides	Non-protein N	Urea N	Preformed Creatinine	Creatinine + Creatinine	Uric Acid	Ca	P	Sugar			
	K.	cc.		cc.N/10 NaOH	gm.	gm.	mg.	mg.	gm.	gm. NaCl	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.			
1923 Dec. 8 11 13	19.0 18.3 18.3	580 290 300	1.020 1.032 1.031	262 182 190	6.23 6.39 6.59	4.91 4.87 4.52	517.4 415.0 500.0	97.4 115.0 0	1.114 1.025 1.03	1.818 1.56 1.5	33.3 31.3	13.6 12.2		1.0	2.9 2.8			102. 83.6			
					Dark period beginning December 15																
18 Jan. 12 15 17	18.3 19.1 19.1 19.1	340 340 355 475	1.045 1.032 1.031 1.35	260 226 235 255	14.98 6.76 5.14 5.18	12.6 4.01 3.56 3.47	500.0 275.0 410.0 600.0	440.0 250.0 95.0 165.0	1.315 1.115 0.870 0.87	2.2 2.36 2.29 3.16	69. 30.7 38.1	14.4 20.2	0.9 1.8 1.9	2.9 2.2 1.5	1.7 2.1	11.9	4.9	82.8 77.8 90.8			
					Light period beginning January 21																
22 24 26 31	19.1 19.3 19.2 19.2	470 275 315 360	1.03 1.042 1.025 1.025	475 293 200 105	17.5 9.52 9.51 7.46	15.23 7.28 8.12 5.74	600.0 630.0 473.0 476.0	300.0 135.0 0 49.0	2.11 1.135 1.145 0.905		63.5 30.3 29.	27.4 16.6 10.1	2.6 1.6 1.6	0.6 1.9 1.3	1.7 1.8 2.2	11.7 10.8	4.8	109.1 103.4 105.			
Dog G																					
1923 Dec. 11 14	12.7 13.0	530 430	1.014 1.017	176 168	5.67 5.25	3.56 3.86	340 317	130.0 115.0	0.63 0.17	1.57 1.53	31.1	9.8 10.6	1.8 2.0	2.4	3.0 2.8			97.3 126.			
					Dark period beginning December 15																
18 Jan. 12 17	13.0 14.1 14.1	250 300 175	1.025 1.033 1.038	178 258 193	4.98 6.51 4.62	4.14 3.73 4.12	336 320 323	260.0 110.0 0	0.545 1.145 0.91	1.48 1.55 2.64	42.3 31.6 31.6	12.6 16.7	1.0 2.4 2.8	2.8 1.9 0.9	3.9			126.8 96.6 141.8			
					Dark period ending January 21																
22 24 26 31	14.4 14.3 14.2 14.2	175 190 165 150	1.037 1.039 1.043 1.04	163 228 173 140	4.42 5.98 5.5 4.13	3.27 3.95 4.10 3.70	325 450 333 370	175.0 190.0 0 82	0.92 1.165 0.925 0.88	1.53 1.37 1.63 0.99	34.5 31.6 30.5	16.8 13. 11.	1.8 1.6 1.7	2.6 3.3 3.2	2.0 2.3 2.4			155.4 109. 93.			

85 (2608)

Histo-chemical proof of the presence of protein matter in dental enamel.

By CHARLES F. BODECKER and WILLIAM J. GIES.

*[From the Laboratories of Histology and Biological Chemistry,
School of Dentistry, Columbia University.]*

There has been much speculation regarding the occurrence of protoplasmic matter in dental enamel but no demonstration of it, although recent histological work, by Bödecker particularly, has suggested the presence of organic structural elements. In a direct histo-chemical study, human teeth, after immersion for several days in 10 percent formalin solution, were sectioned transversely through the crowns. The sections were ground to the thickness of about 1 mm. or less; decalcified by Bödecker's process¹ in a mixture of methyl alcohol, nitric acid, and celloidin; freed from celloidin with a mixture of ethyl alcohol and ether, and kept in ethyl alcohol. Throughout this procedure the residual structure of the enamel retained the original outlines. A portion of the soft yellowish-white enamel residue, about a millimeter wide and several millimeters long was gently teased away, transferred carefully with a pipette to water, in which it was soaked for the removal of soluble extraneous matter; and then pipetted cautiously to a microscope slide, where the identity of the material was confirmed and absence of dentinal admixture established. At this stage the particle was slightly acid in reaction to litmus. The quantities of this material in dental enamel differ widely.

The fragment of soft enamel residue, which remained intact during these manipulations, was then treated on the slide with a few drops of Gies's reagent for Piotrowsky's ("biuret") test. Gradually the particle under microscopic examination acquired a lavender tinge of uneven intensity, the coloration ultimately becoming evident to the naked eye. This result was obtained as well with fully as with incompletely decalcified specimens. The Molisch test for carbohydrate, and the ammoniacal silver test for aldehydes, applied to other pieces, were negative, indicating that

¹ Bödecker: Proceedings of the New York Section of the International Association for Dental Research, and of the New York Academy of Dentistry, *Journal of Dental Research*, 1925 (in press).

the result with Gies's reagent was due neither to a reducing substance derived from the celloidin nor to retained formalin.

Since no insoluble material except a protein mass gives this color reaction, under the severely exclusive conditions of this test (including the prolonged initial treatment of the original tooth with formalin and of the sections with nitric acid, alcohol, ether, and water), the result, repeatedly obtained, presents conclusive histo-chemical evidence of the presence of protein in enamel, and of the occurrence there of true protoplasmic (organic) matter. This general finding is directly corroborated by the fact that pieces of the enamel residue, when gently warmed in Millon's reagent, give the red response that is typical of insoluble protein matter.

The enamel protein is stained deeply by trypan blue. Gies demonstrated some years ago that *while dental tissue is being produced, injected trypan blue* is absorbed into the enamel, from the circulation of dogs, and is permanently retained; but that trypan blue does not pass into the enamel *after* its construction.² (A dog nearly eight years old showing such retention was presented.) Since injected trypan blue, after staining the tissues, is removed from all parts of the body except dental enamel (after its passage into enamel under the special conditions just stated), it is probable that the degree of true *nutritional* transformation, as distinguished from simple osmotic change, in enamel *after its production*, is very slight if not wholly negative.

Further histo-chemical study in this relation may be expected to throw new light not only on the distribution and probable structural relationships of the protein matter in enamel, but also on the origin and prevention of dental caries.

² Gies: *Journal of the National (American) Dental Association*, 1918, v, 529.

86 (2609)

Histological changes in the testis of the Guinea pig during scurvy and inanition.

By BLANCHE LINDSAY and GRACE MEDES (Introduced by
J. F. McClendon).

*[From the Laboratory of Physiologic Chemistry, University of
Minnesota, and the Physiology Laboratory, Wellesley
College, Wellesley, Mass.]*

The histological changes in the testes of guinea pigs fed a diet deficient in vitamin C were studied and compared with the conditions in guinea pigs of different ages on normal diet. The conditions in guinea pigs were also studied when the diet contained the three vitamins but inadequate food supply. The effect of the two deficient diets was to cause retardation of development in young animals, and degeneration of spermatozoa, spermatids and spermatocytes in older animals. In less severe cases only the early stages of spermatogenesis were affected. Signs of degeneration consisted of desquamation of germinal epithelium into the lumen of the tubules, presence of degenerating cells taking a deep acidophilic stain, and disintegration of the cytoplasm producing a reticular appearance. Sertoli cells were not affected. There was no hypertrophy of intertubular tissue or of interstitial cells. Variation in the amount of change in the testis was found to be parallel to that in the adrenal glands, and both seem to depend on the size of the animal and on the length of time it had been fed a scorbutic diet. Guinea pigs fed a normal diet after chronic scurvy possessed testes containing germ cells in all stages, although the proportion of tubules containing spermatozoa was considerably reduced.

87 (2610)

The mechanism of shortening of the lag period in bacterial cultures containing certain food accessory substances.

By GREGORY SHWARTZMAN.

[From the Laboratory of Bacteriology, N. Y. H. Medical College, and Flower Hospital, New York City.]

Avery and Morgan¹ have recently demonstrated that fresh potato tissue, which was shown to contain a powerful peroxidase as well as certain food accessory substances, is able to promote the growth of pneumococcus in culture media. In these studies they noticed that a piece of potato when added to a broth culture of pneumococcus is able to abolish the lag period in the growth of this micro-organism, which enters, immediately after inoculation, into the logarithmic phase of growth.

In the author's experiments, the influence of tomato extract on the lag period in *B. Shiga* cultures was studied. It was found that this extract is able to shorten the lag period in cultures of this micro-organism if the original inoculum is sufficiently large.

The object of this investigation was to find an explanation of the mechanism of the shortening of the lag period by tomato extract. For this purpose a clear understanding of the function of the lag period in general is required. This seems to have been given by Sherman and Albus.² These authors believe that during the lag period the old cells of the inoculum undergo a biological rejuvenescence which fits them for reproduction. As a matter of fact, they showed that, just before the end of the lag period when no measureable increase in growth had as yet taken place, the inoculated old bacterial cells assumed certain characteristics which belong to young and actively multiplying bacteria. This was shown in the fact that old bacteria inoculated into broth remained resistant to the action of a 5 percent NaCl solution for a period of about one hour and became susceptible, *i. e.*, were killed by the NaCl after 1½ hours of inoculation, or just before the end of the lag period. This increased susceptibility

¹ Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1924, xxxix, 275 and 289.

² Sherman, J. M., and Albus, Wm. R., *J. Bact.*, 1924, ix.

of the bacterial cells to 5 percent NaCl, they take as evidence of rejuvenescence.

Since the lag period seems to be strictly associated with the time required for the bacterial cells to assume the characteristics of young cells, it may be assumed, *a priori*, that any factors which are able to induce a prompt "rejuvenescence" of bacterial cells, in terms of susceptibility to 5 percent NaCl, should bring about a shortening of the lag period. As has been stated above, the author found that the tomato extract is able to shorten the lag period. He concluded that the food accessory substances of this extract are able to "rejuvenate" the bacterial cells in a very short time, make them fit for reproduction much quicker than would occur in plain broth, and bring about the shortening of the lag period.

The following experiments were carried out:

1. The resistance to hypertonic salt solution of *B. Shiga* when exposed to the influence of tomato extract for various intervals of time, was determined, to discover whether a prompt "rejuvenescence" actually occurs.

2. A study was made to determine whether this "rejuvenescence" occurs at the time when no increase in the amount of cells had as yet taken place.

3. An investigation was made to determine whether the same factors, which are responsible for the shortening of the lag period, are also responsible for the "rejuvenescence" of the bacteria.

The technique of testing the resistance of bacterial cells to the action of 5 percent salt solution was as follows:

A four day old culture of *B. Shiga* was inoculated into media of known hydrogen ion concentration. Immediately after seeding, and at 15 minute intervals thereafter, 0.1 cc. of the culture was removed, mixed with 9.9 cc. of salt solution, and plates inoculated for counts of viable micro-organisms. The salt solution suspensions were then kept at room temperature for fifteen minutes and plates again inoculated for counts of viable organisms. The percentage of micro-organisms left alive after treatment with this solution for fifteen minutes was considered as the *index of resistance*.

In preliminary experiments it was found that an inoculum of *B. Shiga* derived from a four day old culture, and freshly

seeded in *plain* broth, reached an index of resistance of 38-40 in *one hour and five minutes* of incubation. This is coincident with the end of the lag period.

Inoculations were now made with tomato extract broth of the same pH as was used in the plain broth (pH 7.0), and it was found that the index of resistance 38-40 was reached in this medium in *15 minutes*. (Fig. 1.) This index, as is seen from

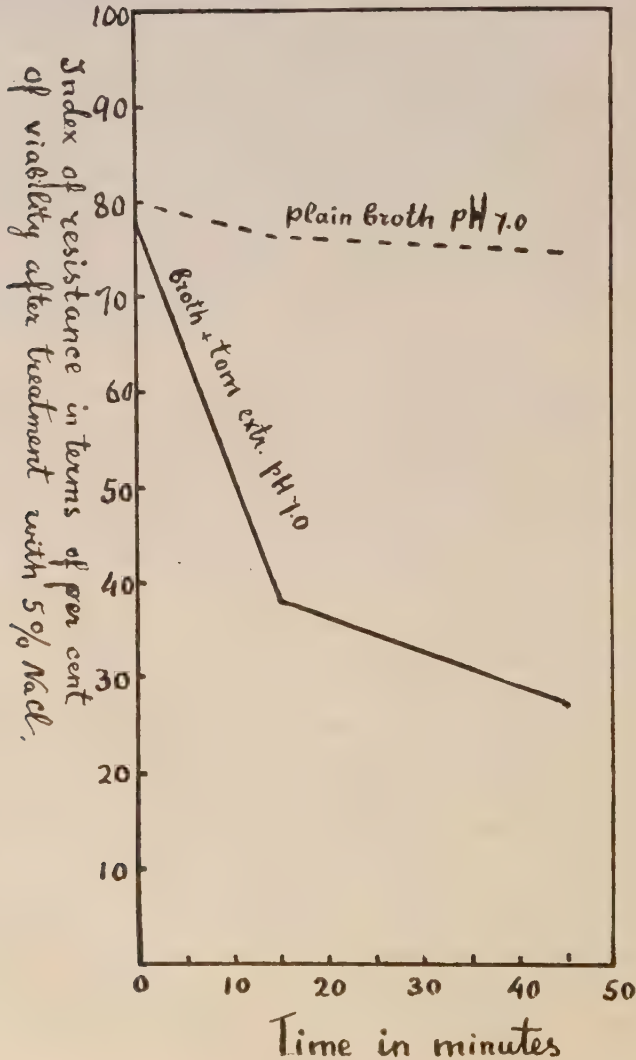


FIG. I.

the above experiment, seems to be necessary in order that the bacteria may enter the phase of logarithmic growth.

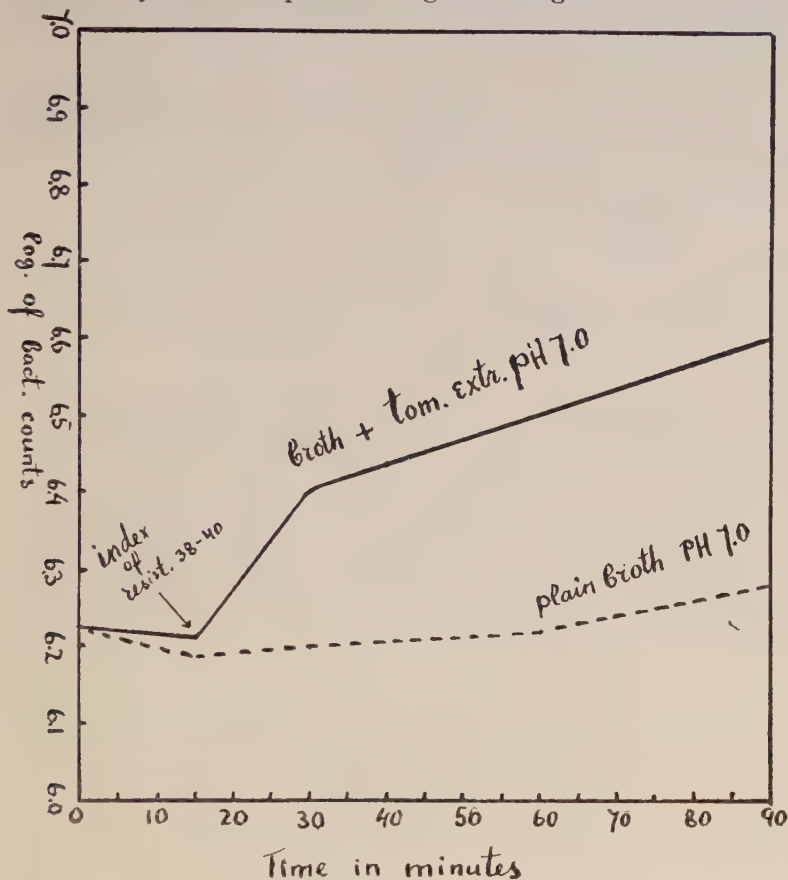


FIG. II.

Fig. II shows the rate of growth of the same inoculum which was employed for the above experiment. This curve again demonstrates that the lag period in the culture containing tomato extract has been shortened to 15 minutes. Moreover, a comparison of Figures I and II brings out the fact that the tomato extract cultures of *B. Shiga* reached the index 38-40 just before an appreciable increase in the numbers of viable micro-organisms.

Tomato extract broth culture from which the food accessory substances were removed by a method described elsewhere³ were inoculated with *B. Shiga*. This experiment demonstrated that in

the absence of growth promoting factors there is no change in the resistance of *B. Shiga* to 5 percent salt solution for a period of 45 minutes of incubation.

To sum up, the food accessory substances of tomato extract are able promptly to "rejuvenate" *B. Shiga*, and thus make it possible for this micro-organism to enter the phase of active multiplication very quickly after inoculation.

88 (2611)

Acetylation as a physiologic reaction.

By C. P. SHERWIN.

[*From the Laboratory of Biochemistry, Fordham University, New York City.*]

After the ingestion of para-amino phenyl acetic acid by human beings it was found that this substance was detoxicated by acetylation of the amino group. When fed to rabbits, acetic acid was also employed as the detoxicating agent and p-acetyl amino-phenyl acetic acid was excreted in the urine. When the p-amino phenyl-acetic acid was fed to dogs, however, the amino group remained free and the detoxication of the compound was accomplished by combining with glycocoll.

Ortho, meta and para amino benzoic acids were ingested by human beings then fed to dogs and rabbits. All three compounds were excreted unchanged in the urine of the dog. The ortho amino benzoic was excreted unchanged by the human being and rabbit, but the meta and para amino benzoic were acetylated by the human being as well as by the rabbit. Ortho acetyl amino benzoic acid was prepared and fed to the dog and rabbit as well as taken by a human subject. The acetyl derivative was found to be non-toxic, and was in each case rapidly excreted unchanged. There was in no case a glycocoll compound formed as reported by Salkowski¹ after feeding meta amino benzoic acid to a human being, dog and rabbit.

³ Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii, 44.

¹ Salkowski, *Zeit. Physiol. Chem.*, 1882-83, vii, 93.

Minnesota Branch.

University of Minnesota, Minneapolis, Minn., November 5, 1924.

89 (2612)

The inverse relation of iodine and goiter in Utah.

By J. C. HATHAWAY (Introduced by J. F. McClendon).

[From the Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.]

Various writers have shown statistically and by analyses the relation that iodine starvation bears to goiter, both simple and exophthalmic. McClendon and Hathaway¹ showed that Utah lies in the border line area of a goiter map of the United States, and the writer thought that it would be of value, therefore, to determine the relation of iodine contained in the water supply to goiter, in different localities of this State. The State Board of Health of Utah has been very cooperative in sending the results of surveys of the goiter situation in school children from places from which water samples were obtained. The following table shows rather strikingly the inverse relation of the iodine in the water supply to the goiter situation in these localities.

Town	Iodine in parts per hundred billion	Goiter percent
Lakeview	216	6
Goshen	250	15
Santaquin	61	45
Alpine (2 analyses)	18	57
Provo	30	High (exact per cent not given)

¹ *J. Am. Med. Assn.*, 1924, lxxxii, 1668.

90 (2613)

The probable occurrence of xerophthalmia in turkeys.

By GEORGE C. McCLENDON, (Introduced by J. F. McClendon)
Linden, Calif.

Some experiments which I have made on turkeys suggest that an eye disease is cured and prevented by diet. From an analogy to experiments on mammals and chickens, it seems probable that this disease is xerophthalmia. Since these experiments are of an expensive nature, it is considered that this information may be worth while to others working in this field.

In 1922, ten turkeys hatched from the egg developed ophthalmia. Five of them with ophthalmia were fed full cream cheese as an exclusive diet, and got well very quickly; the five in the control got worse and died. In 1923, 250 turkeys hatched from the egg were fed cream cheese, egg, cornmeal and egg shell. The cheese and egg yolk were considered a preventative. No ophthalmia developed. Of three controls, one gobbler developed ophthalmia and was cured by the cheese diet. The only measure taken against infection of the turkeys was keeping them several miles away from any other turkeys or other domestic animals.

91 (2614)

An immunological and chemical study of the alcohol-soluble proteins of cereals.

By JULIAN H. LEWIS, H. GIDEON WELLS, WALTER F. HOFFMAN
and ROSS AIKEN GORTNER.

[From the Otho S. Sprague Memorial Institute, the Department of Pathology of the University of Chicago and the Division of Agricultural Biochemistry, University of Minnesota.]

The prolamines, or alcohol-soluble proteins, are the characteristic proteins of cereal grains. These proteins were isolated from wheat, *Triticum vulgare*, durum, *Triticum durum*, emmer, *Triticum dicoccum*, spelt, *Triticum spelta*, einkorn, *Triticum monococcum*, rye, *Secale cereale*, oats, *Avena sativa*, barley, *Hordeum vulgare*, corn, *Zea mays*, kafir, *Andropogon sorghum*, teosinte, *Euchlaena mexicana* Schrad., and sorghum, *Sorghum vulgare*, and subjected to chemical and immunological study.

The chemical study included the nitrogen distribution by the Van Slyke method, the free amino nitrogen, the free carboxyl groups, the true ammonia nitrogen, the cystine and tryptophane content, and the acid and alkali binding at various hydrogen ion concentrations and at different temperatures. This study showed certain similarities of chemical composition among the prolamines, as a class, as contrasted with the composition and behavior of such proteins as casein and fibrin. The chemical evidence suggested that the prolamines studied might be grouped into a "wheat group", which would include the proteins isolated from the genus *Triticum*, and a "corn group" including those isolated from maize, teosinte, kafir, and sorghum.

The genetic behavior of these groups has been extensively studied by plant breeders, although relatively more work has been done upon the wheat group. Sakamura,¹ Kihara,² and Sax³ have shown that *T. monococcum* is characterized by having 7 chromosomes, that *T. dicoccum* and *T. durum* have 14 chromosomes, and *T. vulgare* and *T. spelta* have 21 chromosomes. The fertility or

¹ Sakamura, *Bot. Mag.*, Tokyo, Vol. 32, 1918 (cited by Sax, *loc. cit.*).

² Kihara, *Bot. Mag.*, Tokyo, Vol. 33, 1919, Vol. 35, 1921 (cited by Sax, *loc. cit.*).

³ Sax, K., *Science*, 54, 413-15, 1921.

total or partial sterility of inter species crosses is in accordance with these chromosome numbers. In the "corn group" Collins⁴ has shown that teosinte will hybridize with *Zea mays*, indicating a genetic relationship.

Because of these observations it was believed that the immunological relationships of these proteins would prove of interest. Proteins of the "wheat group" were accordingly compared with proteins of the "corn group" and with each other, by means of the complement fixation test and by the anaphylaxis test, using the uterus strip method, the bronchospasm method, and the guinea pig reaction.

Complement fixation test:

Rabbits were immunized by repeated injections of gliadin, glutenin, and zein. Each of the three antisera obtained was tested against homologous protein and all the remaining proteins in amount of 0.01 mg., 0.1 mg. and 1.0 mg. The reactions brought out very clearly the relation of the proteins of the grasses to the proteins of wheat and corn. The prolamines from emmer, einkorn, spelt and durum are closely related to gliadin and glutenin from *T. vulgare*, while those from teosinte and kafir are closely related to zein from *Zea mays*. The former are more closely related to gliadin than they are to glutenin, while the protein from teosinte is more closely related to zein than is kafirin. No reactions were obtained between antisera for the corn group with proteins from the wheat group, or conversely.

Uterus strip method:

The results show the very close relationship of gliadin, and the proteins of emmer, einkorn, and spelt. A uterus strip that is sensitive to any one of these proteins is also sensitive to all of them. When the strip is desensitized to any one it is also desensitized to the others. A uterus strip sensitized to the protein of teosinte will contract and be completely desensitized to teosinte protein by exposing it to zein or to kafirin.

Bronchospasm method:

This is a new method described by Koessler and Lewis,⁵ which permits graphic tracings being made of the bronchospasm occurring in guinea pigs as a specific reaction to proteins to which the

⁴ Collins, G. N., *J. Heredity*, 12: 339-50, 1921.

⁵ Koessler and Lewis, in press.

guinea pigs are hypersensitive. The effects produced with the prolamines correspond completely to those observed by the uterus strip method.

Guinea pig anaphylaxis tests:

Experiments were made in which guinea pigs were sensitized with wheat gliadin or with zein, and then after two weeks given intro-peritoneal injections of the different prolamines. Although this method is not so delicate as the uterus strip and broncho-spasm methods, the results were quite the same, showing practical identity of proteins in the two groups. That is, guinea pigs sensitized with gliadin gave strong reactions when reinjected with the other four proteins of the wheat series, but none at all with proteins from the corn series. Animals recovering from these crossed reactions with the proteins of the wheat series, were protected against gliadin just as were the tissues in the uterine strip experiments.

Therefore we have data obtained by four different immunological methods all giving identical results, so that they may be looked upon as conclusive.

The detailed reports of these experiments will appear as separate publications. The chemical and physico-chemical data will appear in the Second Colloid Symposium Monograph to be issued by the Chemical Catalogue Company of New York City. The immunological data will probably appear in the Journal of Biological Chemistry.

92 (2615)

Further studies on the etiology of epidemic hiccough.

By EDWARD C. ROSENOW.

[From the Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.]

In two previous epidemics I have isolated a streptococcus from infection atriæ in cases of epidemic hiccough which produced spasms of the diaphragm and other muscles in animals. This property was readily lost on ordinary aerobic cultivation, but by

rapid transfer (four to eight times in twenty-four hours). In partial tension cultures, its specific infecting power was maintained through as many as fifty culture generations. Moreover, when the living streptococcus incited spasms of the diaphragm, the washed dead bacteria and filtrate of the actively growing culture also produced this phenomenon. This streptococcus had the power of producing a poison which caused lesions in the cervical cord or in the roots of the phrenic nerve, and provoked spasms of the diaphragm.

In a recent outbreak of another epidemic, I have again isolated this streptococcus. The symptoms in the present epidemic are less severe, more irregular and of shorter duration than those of the two previous epidemics studied. The duration of hiccough in the six cases studied ranged from two to eight days. In five of the six there was an associated infection of the throat, of varying severity. Moderate nasopharyngitis or tonsillitis was found in all. Cultures on blood agar plates of nasopharyngeal swabbings and of pus expressed from tonsils showed an unusually large number of green-producing streptococci, often in almost pure culture. Precipitin tests with nasopharyngeal washings and my poliomyelitis and encephalitis serums were positive in three and negative in three of the cases.

Of the thirty-two animals injected intracerebrally with material from infection atria or partial tension cultures thereof, fifteen were seen to have spasms of the diaphragm; thirteen, of the abdominal muscles, and twenty-three, of other muscles. A total of twenty-eight had spasms of the diaphragm or of other muscles. The same streptococcus isolated in previous epidemics was isolated from brain and cord of eighteen positive animals, usually in pure culture. One of these strains was rapidly transferred through ten culture generations. Small amounts of the living culture and larger amounts of the dead bacteria, and corresponding filtrates, still produced spasms of the diaphragm or of other muscles.

Effects such as these were rarely encountered during a large number of similar experiments in other diseases since the previous epidemic of hiccough.

93 (2616)

A precipitating and neutralizing antistreptococcus (scarlatinae) horse serum.

By EDWARD C. ROSENOW.

[From the Department of Experimental Bacteriology, Mayo Foundation, Rochester, Minnesota.]

The immune serum, used in the experiments to be reported, was prepared in the horse (Horse 10) by repeated inoculation of hemolytic streptococci freshly isolated from the acutely inflamed throats of three typical cases of scarlet fever. The injections were made intravenously on three successive days each week from December 19, 1917, to April 20, 1918. The initial injection consisted of the heated (56° for forty-five minutes) bacteria from 100 cc. of glucose broth, suspended in 10 cc. of sodium chloride solution. The heat-killed organisms were injected during the first five weeks, and live bacteria during the remainder of the period of immunization. The dose was gradually increased until the organisms from 600 cc. of broth were given at a single injection. The horse remained well until the latter part of March, when it developed arthritis which gradually increased until May 6, when it was bled to death under anesthesia. The serum used was obtained from this bleeding and from bleedings made April 8 and May 1. After the serum had been proved sterile, 0.2 per cent tricresol was added, and the serum placed in amber bottles securely stoppered and kept continuously in the ice chest.

Another horse (Horse 19), whose serum was used as a control, was immunized in a similar manner but with hemolytic streptococci from septic infection.

The small epidemic of scarlet fever which occurred in Rochester when inoculation of the horse was begun, had disappeared before immunization was considered adequate to give the serum therapeutic value, and hence it was not used in treatment. However, it was found to have marked agglutinating power over the strains inoculated as well as over other strains from scarlet fever, but little or no effect on a few strains of hemolytic streptococci from other sources.

PRECIPITIN EXPERIMENTS.

The precipitin tests consisted of layering various cleared extracts of the streptococci over the respective serums in small glass tubes (3.5 cm. long by 3.5 mm. inside diameter), and noting the presence or absence of a cloud at the junction of extract and serum, after two hours' incubation and after being kept in the ice chest overnight. The readings were made in a darkened room by transmitted light, obtained from below the shade of an electric light bulb, directing the eye into a dark background.

In my work on specificity in the group of green-producing streptococci, it was found that specific agglutinating and precipitating properties were maintained over long periods when freshly isolated strains were kept in dense suspension in glycerin (two parts) and 25 per cent sodium chloride solution (one part). The glycerin-salt solution extracts used in the experiments were made by diluting such dense suspensions of hemolytic streptococci with water, to the density of the original broth culture, and centrifuging until water-clear. The extracts from the old blood-agar slant cultures were made by adding 2 cc. of distilled water, slanting the tubes so that the water covered the slant, and leaving the tubes over night in the ice chest. The extracts from the throats were made by swabbing the nasopharynx, washing the swab in

Precipitin reactions with antigens from various hemolytic streptococci.

Source of hemolytic streptococcus	Source of Antigens	Number of strains tested	Number of positive reactions with serum from		
			Horse 10 (Scarlet fever)	Horse 19 (Septic infection)	Normal horse
Scarlet fever	Glycerin-salt solution extract	18	17	0	0
Scarlet fever	Glucose-broth culture	12	9	0	0
Scarlet fever	Extract in water of old blood agar slants	5	5	1	0
Miscellaneous	Glycerin-salt solution extract	12	2	4	0
Scarlet fever	Sodium chloride solution suspension of nasopharyngeal swabbings	32	17	1	0
Normal controls	Sodium chloride solution suspension of nasopharyngeal swabbings. Persons not exposed to scarlet fever.	76	3	0	0

2 cc. of sodium chloride solution, squeezing out the fluid from the cotton, and centrifuging until water-clear.

The results obtained with extracts of hemolytic streptococci from scarlet fever and other sources, and with extracts of nasopharyngeal swabbings in cases of scarlet fever and in normal controls, are summarized in Table 1. Only negative results were obtained with immune serums used as controls, which were prepared by injecting green-producing streptococci isolated respectively in poliomyelitis, encephalitis, chorea and influenza, and with antipneumococcus serums.

The results of the precipitin reaction are in agreement with those of Tunnicliffe,¹ Bliss,² Dochez³ and others, in that they show the hemolytic streptococcus from scarlet fever to be quite homogenous, and that hemolytic streptococci from other sources are more heterogenous. Nearly all in the former group reacted positively with the immune scarlet fever serum, while only four of the twelve strains from other sources gave a positive test with the immune serums prepared with the hemolytic streptococcus from septic infection. The hemolytic streptococcus was demonstrated in the nasopharyngeal swabbings in all but one of the seventeen cases of scarlet fever that yielded a positive precipitin test, whereas in the fifteen that reacted negatively, it was found in only three. All tests were positive in the acute stage of the disease.

The reaction appears to be specific, and should prove useful in classifying hemolytic streptococci, and in differentiating between scarlatinal and non-scarlatinal throat infections, and hence be of diagnostic value in scarlet fever.

NEUTRALIZATION EXPERIMENTS

The technique of our neutralization experiments was similar to that developed by the Dicks.⁴ The respective organisms were

¹ Tunnicliffe, Ruth: The specific nature of hemolytic streptococcus of scarlet fever, *J. Am. Med. Assn.*, 1920, lxxxii, 1396.

² Bliss, W. P.: Abiological study of hemolytic streptococci from throats of patients suffering from scarlet fever, *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 173.

³ Dochez, A. R.: Studies concerning the significance of streptococcus hemolyticus in scarlet fever. *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 194.

⁴ Dick, G. F., and Dick, Gladys H.: A skin test for susceptibility to scarlet fever. *J. Am. Med. Assn.*, 1924, lxxxii, 265.

grown in human or horse blood broth for four to seven days, and the cleared supernatant broth culture was passed through bacterial filters (Mandler type). The neutralizing power of the immune scarlatinal serum and control serums was tested by mixing equal parts of the serum with the filtrate or dilutions thereof, incubating for one hour, and injecting 0.2 cc. into the skin over the forearm of humans. Severe initial reactions, due to the toxicity of the filtrates; delayed urticarial reactions, due to the horse serum; the danger of rendering persons sensitive to horse serum; and the extreme variations in susceptibility of humans, emphasized the importance of finding a suitable test animal to determine the neutralizing power of this and the newly developed therapeutic scarlatinal horse serums. Many animals were tested. The horse, cow, goat, dog, monkey (*Macacculus rhesus*), rabbit, guinea pig, white rat, mouse and fowl were all found insusceptible to intracutaneous injection. The skin over the abdomen of pigs (Chester white) weighing from 20 to 50 pounds, and the skin in the groin and axilla of the sheep and lamb was found to be susceptible.

The reaction in these animals is sharply defined, but reaches its height in about eight hours, instead of twenty-four hours as in man, and the secondary reaction some days later is uncommon. Pseudo reactions following control injections of uninoculated blood broth, filtrates, normal and control serums rarely occur. The dilution of filtrate should be about one-tenth as great as for humans. Parallel toxicity and neutralization experiments in man, pig and sheep gave virtually the same results, although those in the pig were the most uniformly satisfactory.

It has been found that the toxicity of filtrates varies greatly according to the culture medium used, blood-broth cultures yielding filtrates of highest toxicity. Fresh kidney tissue in parallel experiments markedly reduced the toxicity. In one series of experiments, all but two of thirteen scarlatinal strains yielded filtrates of high toxicity. The two yielding only slightly toxic filtrates were isolated, one four, and the other three and a half years previously. In this same experiment, six of eleven non-scarlatinal strains yielded filtrates of high toxicity whose action was indistinguishable from that of the scarlatinal strains. Control strains of green-producing streptococci never produced filtrates manifesting high toxicity on intracutaneous injection. The immune scarlatinal serum from Horse 10, had marked neutraliz-

ing power, not only over nearly all filtrates from scarlatinal strains, but also over those from non-scarlatinal strains. The serum from Horse 19 had slight neutralizing power over some filtrates; normal horse serum had none. The identity of the toxin produced by the respective strains is further shown by the fact that convalescent scarlet fever serum, and the serum of humans after severe reactions to inoculations of scarlet fever filtrates, had neutralizing power over both sets of filtrates.

94 (2617)

A study of the occurrence of peptide nitrogen in the blood.

By WM. W. SWANSON (Introduced by F. W. Schlutz).

[*From the Laboratory of Physiological Chemistry and Pediatrics of the University of Minnesota, Minneapolis, Minnesota.*]

The nitrogen found in tungstic acid blood filtrates, which cannot be attributed definitely to any known nitrogenous substances, has been studied by the following method: To 10 cc. of tungstic acid blood filtrate in a large test-tube graduated to 25 cc. are added 10 cc. of a saturated solution (room temperature) of barium hydroxide. The test-tube is fitted with a reflux condenser, and the contents kept boiling gently for 20 to 24 hours. The solution is then concentrated to 5 cc., a drop of 0.25 percent phenolphthalein is added and sufficient 2 percent sulfuric acid to precipitate the barium. Water is added to the 25 cc. mark, and the contents shaken and filtered. The determination of the free and hydrolyzed amino acids is carried out according to the method of Folin.¹ The difference between the free and total amino-acid nitrogen is considered as peptide nitrogen.

In a recent paper Blau² came to the conclusion that the amount of peptide nitrogen is by far too small to account for the undetermined nitrogen. The values found in the table, obtained by the method described, show that the peptide nitrogen makes up a very high percent of the unknown nitrogen. The reason that a more exact check on the total nitrogen is not obtained, is due probably to unavoidable small losses in the various methods and

¹ Folin, O., *J. Biol. Chem.*, 1922, li, 377.

² Blau, Nathan F., *J. Biol. Chem.*, 1923, lvi, 873.

the variable quantities of the more complex amino-acids, which do not give a color proportional to their nitrogen content. It appears that easily hydrolyzed peptides are responsible for a large percent of the undetermined rest nitrogen.

Non-protein Nitrogen Determinations per 100 cc. of Whole Blood (Human).

No.	Total Non- Protein N	Urea N	Preformed Creatinin N	Total Creatinin N	Uric Acid N	Amino Acid N	Und' ter'd Rest N	Pep- tide N	Peptide N % of Und'ter'd Rest N
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	
1	29	13	0.5	1.6	0.9	8.7	4.8	4.3	90
2	38	17	0.8	1.5	0.84	7.0	11.8	11.0	93
3	45	17.8	0.8	2.0	0.88	8.7	15.6	11.0	70
4	42	11				8.0	23.0	20.0	
5	40	17				8.3	14.7	13.3	
6	40	13.8				7.8	18.4	13.0	
7	43	16.5				7.8	19.0	15.0	
8	36	17.6				9.1	9.3	5.2	
9	41.5	14				8.7	18.8	11.3	
10	142	108	1.8	4.6	1.7	8.7	19.0	13.8	72
11	114	91				8.0	15.0	7.0	
12	115	90			1.6	7.0	16.4	14.0	
13	150	114	3.0		2.0	7.7	23.3	20.0	
14	154	120		3.2	2.6	7.3	21.0	16.0	76

95 (2618)

The effect of sodium ricinoleate upon bacterial toxins, and the value of soap-toxin mixtures as antigens.

By W. P. LARSON, R. D. EVANS and EDMOND NELSON.

[From the Department of Bacteriology, University of Minnesota, Minneapolis, Minn.]

The present study is a continuation of the work published by Larson and Nelson¹ on the effect of castor oil soap upon bacterial toxins. It was shown that sodium ricinoleate possessed the property of detoxifying soluble toxins as well as endotoxins.

In the present paper an attempt is made to analyze the mechanisms of the detoxifying action of soaps, and further, to study the antigenic properties of toxins which have been thus detoxified.

¹ PROC. SOC. EXP. BIOL. AND MED., 1924, **xxi**, 278.

The work was begun by subjecting diphtheritic toxin and tetanus toxin to the action of the sodium salts of a large number of fatty acids as: ricinoleic, oleic, stearic, palmitic, myristic and lauric acids, and two acids with uneven numbered carbon chains, ennenic and hendecenoic acids. Our procedure was to mix the toxin with an equal volume of a one per cent soap solution except in those cases where a one percent solution of soap gels, in which cases weaker solutions were used. It was found that the soaps of oleic, stearic and palmitic acids had no detoxifying action whatever on bacterial toxins. The soaps of lauric, myristic, ennenic and hendecenoic acids possess some detoxifying action upon toxins, but do not compare with sodium ricinoleate in detoxifying properties.

The soaps which detoxify bacterial toxins, differ in physical properties from other soaps, in that they form clear, or nearly clear, solutions in physiological NaCl solution, and dialyze readily through hardened collodium sacs. The detoxifying soaps depress the liquid air surface tension more than do the other soaps of equivalent concentrations used in our experiments.

Early in our work we were impressed by the fact that the detoxifying power of a soap seemed to be in proportion to its solubility in water and inversely with its tendency to gel. It was found that a slightly turbid solution of sodium ricinoleate would not detoxify as effectively as a perfectly clear solution even though the surface tension of the air liquid phase was unchanged. The addition of sodium oleate or sodium stearate to sodium ricinoleate, deprived the latter of its power to detoxify. Colloidal calcium soap, starch, blood serum and nutrient broth have a similar effect. The explanation of this observation may be found in the fact that soap and other surface tension depressants are adsorbed into the surfaces of the colloidal aggregates and other suspended particles in the fluid, and the soap therefore is not free to act upon the toxin molecule. This would lead to the further assumption that the detoxifying action of soap upon bacterial toxin is an adsorption phenomenon. The soap probably dissolves into the surface of the toxin molecule, thus imprisoning it so that it is not free to react with the tissues in the usual manner.

The question naturally arises as to whether the toxin is actually destroyed or merely held imprisoned for a time. In order to shed

some light on this point, very large doses of toxin-soap mixtures were injected into animals. In working with tetanus toxin, we arbitrarily chose as a unit the amount of toxin that would kill a guinea pig, of 400 grams in 24 hours. This was one-half cc. in volume of the particular toxin used. It was found that 0.2 cc. of a one percent solution of sodium ricinoleate was sufficient to completely detoxify this amount of toxin as far as could be determined by guinea pig inoculation. If this amount of soap solution destroys the toxin completely, the guinea pig should be able to withstand multiples of these amounts of soap and toxin without developing symptoms of tetanus. However, when multiples of these amounts were injected, it was found that the animals developed tetanus in periods of time varying with the amount of toxin used. This led us to the conclusion that the toxin molecule is not actually destroyed, but merely imprisoned for a time. The toxin is apparently slowly released at a rate which has not been definitely worked out. When the dose of toxin has not been too large, the release is so slow as not to cause symptoms of tetanus. When the amount of soap-toxin given is greatly increased, the liberation of toxin in a given time is naturally larger, and the animal develops tetanus. The same law holds true of diphtheritic toxin.

The fact that the soap does not change the properties of the toxin molecule is supported by the further observation that both diphtheritic and tetanus toxin which have been detoxified with soap are excellent antigens, as both rabbits and guinea pigs may readily be immunized by treatment with toxin-soap solutions. The serum of rabbits has been found to contain a high titre of antibodies against tetanus toxin after having received four injections over a period of five weeks. Likewise it has been possible to immunize guinea pigs against both diphtheritic and tetanus toxin by this method. We believe that this method of immunization is practical in immunizing humans against diphtheritic toxin. This work is now under way.

96 (2619)

The rate of spore formation in bacteria.

By ARTHUR T. HENRICI.

[From the Department of Bacteriology and Immunology,
University of Minnesota, Minneapolis, Minn.]

The rate of spore formation in *Bacillus megatherium* is shown in Fig. 1, in which the logarithms of the number of vegetative cells and the logarithms of the number of spores are plotted. It will be noted that spore formation is initiated at the end of the active growth period.

I have previously shown that the rate of variation in the size of the cells of *Bacillus megatherium* is dependent in part on the size of the inoculum,¹ in part on the concentration of nutrients in the medium.² I have also observed the influence of these factors on the rate of spore germination and spore formation in *Bacillus cohaerens*. Four sets of cultures were inoculated, two of normal strength agar, and two of quarter strength agar. One set of each kind was seeded with a very heavy emulsion, the other with the same suspension diluted 1-50. The culture used for seeding was an old one, composed almost entirely of spores. The curves indicate the percentage of free spores among 200 cells counted at each time period. The time intervals are plotted on a more extended scale during the period of spore germination than during the period of spore formation. Although the beginning of germination was noted in some spores after an hour, no increase in vegetative cells was demonstrable until the third hour.

It is noteworthy that germination proceeded more rapidly in the lightly inoculated culture than in the heavily seeded one; and commenced more rapidly in the dilute medium than in the full strength agar. The process slowed up more rapidly in the former, and spores never completely disappeared in the heavily seeded dilute medium.

Spore formation proceeds more rapidly in the quarter strength medium, and with the heavier inoculation. This is to be expected, since growth will cease earlier under such circumstances. The actual density of the population in the weaker medium is much

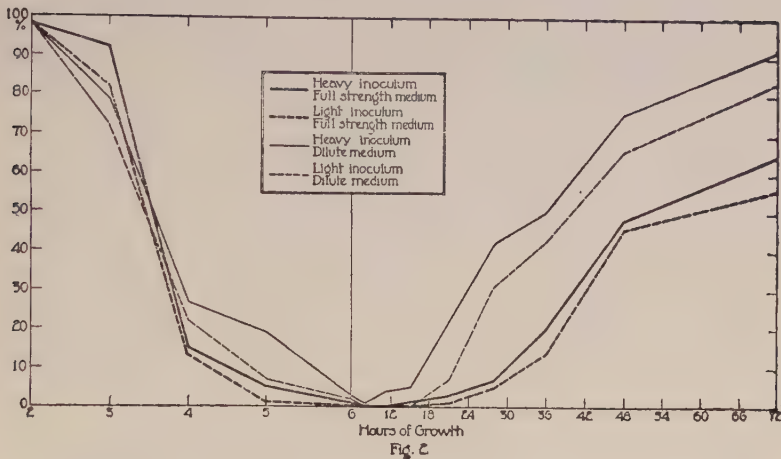
¹ Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 132.

² Henrici, A. T., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 345.

less than in the full strength; therefore the concentration of cells alone is not the determining factor in spore formation.



Fig.1



Peking Branch

Peking Union Medical College, October, 1924.

97 (2620)

The action of ammonia upon the lungs.

By A. A. HORVATH (Introduced by O. H. Robertson).

[From the Chemical Laboratory, Department of Medicine, Peking Union Medical College, Peking, China.]

A study was made of rabbit and guinea pig lungs, and the effect of prolonged inhalation of ammonia in low concentration. The author used for this purpose a special apparatus, giving in the chamber a constant percentage of ammonia. Concentrations of 1.5, 0.5, 0.25 and 0.15 cc. of gaseous ammonia per liter of the chamber air were used. The experimental animals were kept in this chamber constantly until either death occurred or they were killed for examination. Their temperatures were taken daily, and they were weighed from time to time. Out of

a total of 22 experimental animals, 3 rabbits and 11 guinea pigs died spontaneously, and 3 rabbits and 5 guinea pigs were killed. There was a rise of temperature in all the experimental animals exposed to concentrations of ammonia above 0.15 percent, which was usually more noticeable about the middle or toward the end of the period of exposure. The maximum rise was usually 0.3° to 2.5° C. above normal. The clinical symptoms produced were those of a pneumonia. Autopsy and microscopical study showed chiefly catarrhal broncho pneumonia, associated in some cases with fibrino-purulent pleuritis and pericarditis. Lower percentage concentrations of ammonia in the inspired air more often caused pleuro-pneumonia, and higher percentage concentrations only catarrhal broncho-pneumonia. Rabbits were found to be less sensitive to the presence of ammonia in the inspired air than guinea pigs. Guinea pigs were able to tolerate 0.15 percent of ammonia for a month without visible external disturbance or apparent macroscopical changes in the lungs at autopsy; but at 0.25 percent of ammonia gas in the chamber, lesions in the lungs of guinea pigs were noticed. It seems, therefore, that the concentration of ammonia for domestic animals should be lower than 0.25 percent.

The pathological picture in the lungs produced by ammonia, in perfusion experiments or intravenous injection, is quite analogous to that produced by anaphylactic shock. In discussing this phenomenon the author makes the hypothesis that, under the influence of a sensitizing agent, the normal metabolism of ammonia in the organism is disturbed, and the liberated ammonia gives the resulting pathological picture of anaphylactic shock in the lungs. The author found also that habit formation (*Gewöhnung*) for ammonia gas, described by Lehmann and Seifert does not exist. Therefore Lehmann's conclusion that ammonia concentrations are harmless up to 0.5 percent in factory air for "habitual" workmen is open to question.

98 (2621)

Experiments on the administration of tartar emetic by various routes.**By H. JOCELYN SMYLY.***[From the Department of Medicine, Peking Union Medical College, Peking, China.]*

Tartar Emetic is used intravenously in the treatment of Trypanosomiasis, Kalaazar and Schistosomiasis. It has been found by us in the treatment of Kalaazar that the average dose tolerated by human beings is 2 mg. per kilo of body weight given three times a week by intravenous injection of 2 percent aqueous solution. Intravenous injection is difficult in the case of infants, and the following experiments were made to ascertain if a more convenient route would serve in such cases. It cannot be given by mouth on account of its emetic action; subcutaneous injection leads to painful necrosis and possibly ulceration; there remains the possibility of intraperitoneal, of intramuscular and of rectal administration.

Experiments on intramuscular injection were made in four rabbits and one man; intraperitoneal injection in five rabbits; and rectal injection in three patients with Kalaazar.

Intramuscular Injection. Four rabbits were injected with daily doses, ranging in number from one to forty, of 4 mg. per kilo body weight given in various dilutions, some in one place, others at different places at each injection. The rabbits were autopsied and the tissues examined microscopically. Haemorrhage in the muscle followed by necrosis occurred after every injection.

A patient, male, age 22, weight 48 kilos, suffering from Kalaazar, was given 11 intramuscular injections of chemically pure tartar emetic during 46 days, in doses varying from 2 mg. in 4 cc. to 40 mg. in 10 cc. The larger doses gave rise to severe pain and inflammatory swellings:

Conclusion. This method invariably caused pain, swelling and necrosis, and is impracticable for continued treatment.

Intraperitoneal Injections. Five rabbits were given daily intraperitoneal injections of tartar emetic, of varying doses and for

different periods, dissolved in 10 cc. of normal saline per kilo of body weight, as follow:

1. 4 mg. per kilo b. w. for 6 doses: sacrificed.
2. 6 mg. per kilo b. w. for 40 doses: sacrificed.
3. 10 mg. per kilo b. w. for 5 doses: died.
4. 10 mg. per kilo b. w. for 6 doses: died.
5. 20 mg. per kilo b. w. for 2 doses: died.

No. 3 had peritonitis, from which *B. coli* was recovered by culture. Nos. 4 and 5 died of antimony poisoning.

Nos. 1 and 5 showed no peritoneal inflammation. Nos. 2 and 4 showed mild peritonitis, the great omentum being matted in a mass on the greater curvature of the stomach. In No. 2 there were adhesions between the colon and caecum.

Conclusion. This method causes too much irritation to be safely used in children, but may serve in laboratory animals.

Rectal Injection. Three cases were treated daily by this method. After the bowel had been washed out with a plain water enema, 100 cc. of normal saline containing the prescribed dose was injected and was almost always successfully retained. Antimony was detected in the urine in each case but not estimated quantitatively.

Case 1. Male, age 14, weight 18 kilo. Duration of disease three weeks. Symptoms and signs of Kalaazar, but organism not recovered. Doseage 40 mg. to 300 mg. Total 5.78 gm. in 51 days. Result: cured.

Case 2. Male, age 15, weight 31.6 kilos. Duration of disease 1½ years. *Leishmania donovani* obtained by liver puncture and blood culture. Doseage 60 mg. to 1.0 gm. Total 18.44 gm. in 50 days. Disease became worse under treatment. Developed cancrum oris, and was discharged against advice.

Case 3. Male, age 11, weight 22.5 kilos. Duration of disease 1½ years. *L. donovani* obtained by liver puncture and culture. Doseage 0.060 to 0.800 gm. Total 9.6 gm. in 37 days. Disease became worse under treatment. On starting intravenous treatment, showed immediate improvement, which continued for 2 months, following which began to get worse, and antimony treatment was stopped after 4½ months intravenous medication.

Result: The absorption of antimony from the colon in amount insufficient to effect cure apparently produced an antimony-resistant *Leishmania*.

Conclusion: The method fails to cure well-marked cases of Kalaazar and may render them very intractable to further treatment.

The results of this work indicate that intravenous injection is the only practicable route for the administration of tartar emetic in the treatment of human cases of Kalaazar.

99 (2622)

The effect of ephedrine on experimental shock and hemorrhage.

By K. K. CHEN.

[*From the Laboratory of Pharmacology, Peking Union Medical College, Peking, China.*]

In our previous report,^{1, 2} it was shown that ephedrine, an alkaloid isolated from Ma Huang, possesses sympathomimetic actions. Its outstanding feature is its circulatory stimulation by acting on both the cardiac accelerator ganglia and endings. The rise in blood pressure and pulse rate lasts for more than 15 minutes, and sometimes indefinitely.

The purpose of the present paper is to report the influence of ephedrine in hemorrhage and in shock induced by different methods. All the results were obtained from dogs. Histamine in the dosage of 2-3 mg. per kg., or Witte's peptone in dosage of 50 mg. per kg., caused a fall in blood pressure as low as 30-40 mm. hg., but a subsequent intravenous injection of ephedrine always brought it back to the initial level, and maintained it there for more than 3 hours, when the animal was killed. In surgical and traumatic shock, produced by pinching of the liver, exposure and manipulation of intestines, insertion of ice bags into the abdominal cavity, and opening of windows in the operating room, the external temperature being 25° C., the blood pressure in one experiment fell from 150 to 53 mm. hg. within eight minutes, but

¹ Chen, K. K., and Schmidt, Carl F., *Proc. Soc. Exp. Biol. and Med.*, 1924, **xxi**, 351.

² Chen, K. K., and Schmidt, Carl F., *J. Pharm. and Exp. Ther.*, 1924.

was immediately raised to 130 by an intravenous injection of ephedrine. Although there was some fall at the end of 3 hours, it was still well above the critical level. In anaphylactic shock, produced according to the method of Simonds,³ the blood pressure was similarly raised by ephedrine. Finally, the low blood pressure in those animals which had been suddenly bled 25 per cent of their total volume, was quickly elevated by the injection of ephedrine. The beneficial effect in these cases, however, did not last much longer than one hour, on account of much loss of volume. In every case, of either hemorrhage or shock, the pulse rate was permanently increased after ephedrine.

In a total of 22 experiments to date, it was found that, as a rule, 2-3 mg. of ephedrine per kg. of body weight in dogs, seem to be an efficient dose, and that repeated injections give no additional effect although without harmful symptoms. In a few instances, when the heart beat became nearly imperceptible on the pressure tracing, or when the blood pressure stayed too long at a very low level, ephedrine could not raise the blood pressure to any high extent, while the injections of defibrinated blood could still restore it to the normal level.

Although the question of shock is not yet settled, some hint towards the explanation of its cause has been made in connection with the investigation of wound shock by the British Medical Research Committee⁴ during the last World War. The relation of different types of shock is better understood. Dale⁵ explains the fall of blood pressure, in histamine shock, as due partly to the stagnation of blood in the dilated capillaries, and partly to the loss of plasma through the abnormally permeable endothelium. The effective currency is greatly reduced, and the heart is practically working with nothing. Under certain conditions, surgical and traumatic shock is apparently due to nothing but to the absorption of histamine or histamine-like substance from the injured tissues after autolysis, as illustrated by Bayliss and Cannon.^{6, 7} In Witte's peptone, Abel⁸ has shown that the

³ Simonds, J. P., *J. Infect. Dis.*, 1916, **xix**, 746.

⁴ *Br. Med. Res. Com., Sp. Ser.*, 1919, Nos. 25-27.

⁵ Dale, H. H., *Harvey Lectures*, 1919, 20, 26.

⁶ Bayliss, W. M., *Br. Med. Res. Com., Sp. Ser.*, No. 26, 23.

⁷ Cannon, W. B., *Ibid.*, No. 26, 27.

⁸ Abel, J. J., and Geiling, E. M. K., *J. Pharm. and Exp. Ther.*, 1924, **xxiii**,

most toxic element is histamine; and Underhill and Ringer⁹ have already shown the close resemblance between peptone and histamine shock. Suggestion has also been made by Dale¹⁰ that anaphylactic shock follows the same mechanism after the interaction of antibody with the corresponding antigen. Should the question of shock be limited to histamine, and should Dale's idea of histamine shock stand, the explanation of the effect of ephedrine in the cases studied here would be simple.

Of the four main measures of raising blood pressure, the injection of pressor drugs such as pituitrin and adrenalin during shock is useless because the arteries are already constricted to a high degree. The increase in the viscosity of the blood is not practical because it is also increased during shock. One good and sure way of raising the blood pressure is by increasing the blood volume with an homologous blood, and there is no doubt that blood transfusion is one of the best methods, if not the best. Another possible way of raising the blood pressure is by the increase of cardiac contraction; and this appears to be what ephedrine does in shock. In one of our three experiments in which the kidney volume was taken, an injection of ephedrine after histamine caused initial brief constriction, rapidly followed by a continuous dilatation of kidney volume. The blood pressure rose from 24 to 132 mm. hg., and pulse rate to 228 per minute, which persisted for more than 3 hours. Thirty minutes following the injection the kidney was still rapidly dilating. The rise and maintenance of blood pressure can therefore be attributed to cardiac stimulation, and not to arterial constriction. It seems to be fairly clear that the increase in heart contraction and pulse rate *dislodges* the stagnated blood in the capillaries and brings more blood to the effective currency, which a normal heart cannot do during shock. The value of such a mode of action of ephedrine is very essential: (a) It breaks the vicious cycle of shock, (b) it brings more nutrition to the heart itself, the medulla and other essential organs, and (c) it probably hastens the destruction of histamine, for it has been shown that histamine is rapidly destroyed in the presence of efficient circulation.⁶

In case of hemorrhage, ephedrine will be a great help so long as there is hope of restoration of blood volume. If it is not

⁹ Underhill, F. P. and Ringer, M., *J. Pharm. and Exp. Ther.*, 1922, xix, 163.

¹⁰ Dale, H. H., and Laidlow, P. P., *J. Physiol.*, 1918-19, lli, 388.

extensive, ephedrine alone will probably be enough while the blood volume gradually recovers at the expense of tissue fluids. Should it be extensive, it can be used as an emergency drug while transfusion or other important measures are in preparation.

Based on experimental results, it is advocated that ephedrine should be employed in surgical shock and hemorrhage, or as a prophylactic remedy in long operations.

100 (2623)

The experimental transfer of certain intestinal protozoa from man to monkeys.

By JOHN F. KESSEL.

[*From the Parasitology Laboratory, Department of Pathology, Peking Union Medical College, Peking, China.*]

The species identity of intestinal amœbae harbored by monkeys with the intestinal amœbae of man presents a difference of opinion among protozoologists, one group believing them to be identical with the common species found in man, and another group believing them to present distinct species differences. It seems that this question can best be settled by investigation from three main angles, first, by a study of the morphological characteristics, second, by experimental transfer of the protozoa from one species of host to another closely related host, and third, by a comparative study of the various forms under artificial culture conditions.

This paper is a preliminary report of the experimental transfer of certain of the intestinal protozoa from man to monkeys. The routine examination of the faeces of seven monkeys from Southern China, belonging to the genus, *Pithecus*, showed the presence of protozoa indistinguishable morphologically from *E. dysenteriae*, *Endolimax nana*, *Endamoeba coli*, *Councilmania lafleuri*, *Iodamoeba bütschlii*, and *Chilomastix mesnili*. To the knowledge of the writer this is the first report of the presence of *Councilmania* and of *Chilomastix* in monkeys.

Table, showing result of feeding monkeys experimentally with cysts of *Endamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Giardia intestinalis*, and motile forms of *Trichomonas hominis*.

	Monkey No. 1.			Monkey No. 2.			Monkey No. 3.			Monkey No. 4.		
	Protozoa present before feeding	Protozoa fed	Protozoa present after feeding	Protozoa present before feeding	Protozoa fed	Protozoa present after feeding	Protozoa present before feeding	Protozoa fed	Protozoa present after feeding	Protozoa present before feeding	Protozoa fed	Protozoa present after feeding
<i>Endamoeba dysenteriae</i>	+		+	+		+	+		+	+		+
<i>Endolimax nana</i>	+		+	+	+		+	+		+		+
<i>Endamoeba coli</i>	+		+		+		+		+	+		+
<i>Councilmaniana lafeuri</i>							+		+			
<i>Iodamoeba bütschlii</i>					+	+	+	+	+			+
<i>Giardia intestinalis</i>		+			+			+			+	
<i>Chilomastix mesnili</i>		+	+		+					+		+
<i>Trichomonas hominis</i>		+			+	+						

All seven of the monkeys harbored amœbae morphologically indistinguishable from *E. dysenteriae*, and consequently no feeding experiments with *E. dysenteriae* from man could be performed. It should be noted, however, that successful transmission of *E. dysenteriae* from man to monkeys has been reported by Franchini¹ and Ujihara.² Certain of the monkeys of the present series were negative for one or more of the other protozoa in question, and feeding experiments were performed as indicated in the table.

In all cases only a single feeding was made which consisted of five cc. of dilute human faeces containing the protozoa indicated. The routine follow-up examination was made from three to six weeks after the feeding, and the protozoa recovered from the monkeys were morphologically identical with the protozoa fed to the monkeys.

It is seen by this table that infestations of *Endamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii*, *Chilomastix mesnili* and *Trichomonas hominis* of man were successfully established in monkeys which showed no previous infestation with these protozoa.

The ease with which this experimental transfer was accomplished lends evidence in favor of the close relationship and even of the probable species identity of the intestinal protozoa of man and monkeys.

¹ Franchini, G., Experimentelle Tropicdysenterie. Die Entamoeba beim Affen. *Centralbl. f. Bakt., I abt.* (Orig.), 1912, lxi, 590-595, 1 pl.

² Ujihara, K., Studien über die Amöbendysenterie. *Zeitschr. f. Hyg. und. Infes.—Krankheit*, 1914, lxxvii, 329.

SCIENTIFIC PROCEEDINGS.

New York Meeting.

*Cornell University Medical College, New York City,
January 21, 1925.*

101 (2624)

A chemical study of tuberculin.

By J. HOWARD MUELLER.

*[From the Department of Bacteriology and Immunology, Harvard
Medical School, Boston, Mass.]*

Some time ago in the course of a study of the tuberculin and allied reactions, Zinsser¹ showed that extracts of powdered tubercle bacilli, rendered as free as possible from protein, by acid precipitation and boiling, contained substances precipitable by alcohol, which substance produced skin reactions of the delayed type, in tuberculous guinea pigs. The acetic acid precipitate gave a similar reaction, which was not lost on repeated solution in alkali and reprecipitation with acid. Several possibilities of the relationship between the protein precipitate and the so-called "residue antigen," from which the protein had been removed, were discussed and the question was left open for further work.

The investigation of the residue was continued by Zinsser and Parker,² who extended the observations to other bacteria, and found that in the case of each one tried, including staphylococci, pneumococci and meningococci, a similar preparation could be obtained. In the case of these organisms, however, the products were tested by the precipitin reactions, since chronic infections of a type suitable to produce skin hypersensitiveness could not readily be produced in guinea pigs. In all cases these bacterial residues reacted specifically with immune sera by the precipitin test.

¹ Zinsser, H., *J. Exp. Med.*, 1921, **xxxiv**, 495.

² Zinsser and Parker, *J. Exp. Med.*, 1923, **xxxvii**, 275.

It was shown that these preparations were made up largely of non-nitrogenous material, which gave the Molisch test for carbohydrates. Heidelberger and Avery³ have shown that substances prepared from *Pneumococcus* II and III broth cultures, and which are undoubtedly identical with the active material of the residue antigen, are complex carbohydrates or gums. The writer has come to the same conclusion in the study of a specifically precipitable material prepared from bread yeast. The similarity of results from such unrelated types of microorganisms indicates that the production by bacteria of specific gums, reacting with immune sera by the precipitin test, may be a general phenomenon. It should be noted that these substances, while reacting with immune sera, are apparently incapable of themselves inducing antibody formation, and hence are not antigens in the strict sense.

It has been the purpose of the present investigation to determine whether the tubercle bacillus also produces a carbohydrate residue antigen, and if so, whether it is the substance calling forth allergic reactions in tuberculous animals.

Because of the success of Heidelberger and Avery in purifying their pneumococcus material from broth cultures, the material used in the work here reported has for the most part been old tuberculin. Prepared from the usual glycerine infusion broth, this will usually give a precipitin reaction, by the ring test with good immune sera in a dilution of about 1-1000, and a skin test in tuberculous guinea pigs in a dilution of 1-50 to 1-100. In comparing the two types of reaction after various methods of attempted purification, serious discrepancies began to appear. It has finally been possible to show definitely that they are dependent upon entirely different substances in the tuberculin. For example, after several precipitations with alcohol, dilute acetic acid precipitated a substance which gave a strong biuret test, a powerful skin reaction in high dilution, but gave precipitin tests only in dilutions up to 1-1000 of the dry material. On the other hand from filtrate which gave both reactions, due to incomplete removal of the skin reactive material, of the tannic acid precipitation, followed by removal of the excess of the reagent from the filtrate by barium hydroxide, lead hydroxide, etc., a preparation was obtained containing less than 1 percent of nitro-

³ Heidelberger and Avery, *J. Exp. Med.*, 1924, xl, 301.

gen. This gave no skin reaction in a dilution equivalent to the original concentrated tuberculin, but gave a precipitin reaction up to a dilution of 1-40,000.

The tannic acid method, in the form so far used, has served to establish that the skin test and the precipitin test are manifestations of separate and distinct substances in the tuberculin. It, however, causes too great a loss of these substances to be used in quantity production. It was found, that by the cultivation of the organisms on synthetic media, a broth filtrate could be obtained containing both compounds in quantity not markedly inferior to meat broth cultures. From such filtrates it has proved an easy matter, by a single fractional alcohol precipitation, followed by dialysis, to prepare a substance giving the precipitin reaction in a dilution of 1-1,000,000. This material in a dilution of 1-100 gives a doubtful or negative skin test, and is now being collected for further chemical study.

The nature of the component which produces the skin reaction is also under investigation, but beyond the fact that the fraction is largely protein, no definite statement can yet be made.

102 (2625)

Studies on the purification of antibodies. III. Certain methods for the precipitation of pneumococcus protective antibody.

By R. OTTENBERG and F. A. STENBUCK.

[From the Pathological Laboratory, Mount Sinai Hospital, New York City.]

We have tried to apply the principles developed in our work on typhoid agglutinin to the purification of pneumococcus protective antibodies. The task has turned out to be particularly difficult. The great solubility of the pneumococcus makes it impossible to get the primary extracts of sensitized bacteria as free from bacterial substances as in the case of typhoid. The variability in the resistance of mice to pneumococcus infection, and the variable virulence of pneumococcus under culture, make

quantitative observations impractical. For these reasons, we have been unable to get consistent results which could be repeated with regularity. Nevertheless, we did succeed many times in precipitating the pneumococcus protective body in a way which leaves no doubt that the same methods can be applied as were used in the purification of typhoid antibody.

Our primary material was the protective body obtained in alkaline solution by the method of Huntoon, or by a modification which consisted in extracting in N/500 NaOH instead of in $\frac{1}{4}$ percent NaHCO_3 . Precipitation could frequently be brought about without the addition of any metallic salt, by adding hydrochloric acid to a hydrogen ion concentration between pH 4.0 and 4.6. The following protocol is illustrative of these results:

"Isoelectric" Precipitation, and Recovery in the Dissolved Precipitate, of Pneumococcus Protective Antibody.

Ten cc. portions of an alkaline solution of pneumococcus protective antibody were brought to the indicated pH by addition of 0.15 cc. N/40 HCl, and allowed to stand in the ice box overnight. The precipitate was centrifuged, dissolved in N/400 NaOH, and placed on test. Each mouse received 0.5 cc. protective material, and 0.5 cc. 18 to 24 hours broth culture of pneumococcus Type 1, diluted 1-200, *i. e.*, 0.025 cc. of culture. Each dilution was tested on two mice.

TABLE 1.

Material.	Diluted 0		Diluted 1:10	
Original extract	72* S		64 64	
Ppt. obtained at pH 4.0	S S		64 64	
Virulence control cc. of culture	0.000,005	0.000,001	0.000,000,5	0.000,000,25
	64	64	64	64

* The figures indicate the number of hours before death of animals.
S = survival.

The addition of copper chloride, as in the case of typhoid antibody, was found to greatly facilitate precipitation of the antibody. The most favorable strength of copper chloride was about M/2200. A final concentration of M/1100 had a distinct destructive effect on the pneumococcus antibodies.

Copper Precipitation with Recovery in the Precipitate of Pneumococcus Protective Antibody.

Twenty cc. of pneumococcus antibody extract was added to 2 cc. of M/200 copper chloride, and 2.3 N/40 HCl. Precipitate formed, allowed to stand in ice box over night, centrifuged and dissolved in original volume (20 cc.) of N/200 NaOH. Each animal received 0.2 cc. protective material, diluted to 0.5 cc., with saline and the indicated amount of pneumococcus Type 1, 18 to 24 hour culture, diluted to 0.5 cc. with broth.

TABLE 2.

Material	Dose of Culture		Nitrogen per 100 cc.
	0.01 cc.	0.06 cc.	
Original extract	S	16	12 mgm.
	88	40	
Copper precipitate dissolved	S	40	6.6 mgm.
	88	88	
Virulence control cc. of culture	0.000,000,1	0.000,000,01	0.000,000,001
	40	40	S
	40	40	S

This method can be used for the concentration of antibodies as shown in the following protocol:

Twenty cc. pneumococcus protective antibody solution was added to 2 cc. M/200 copper chloride solution, plus varying quantities of hydrochloric acid as tabulated. Precipitates centrifuged off and dissolved in 5 cc. saline and enough normal NaOH solution (2-3 drops) to cause complete solution.

In this protocol the precipitate, dissolved in one-fourth the volume of the extract, protected against ten times the number of fatal doses as compared with the original material.

TABLE 3.

cc. N/40 HCl	Dose of Culture			Nitrogen per 100 cc.
	0.001 cc.	0.01 cc.	0.06 cc.	
1.3	90 S	48 S		
1.5	S S	S S	50 24	15 mg.
1.7	90 S	77 S	24 45	23 mg.
1.9	S S	45 S	24 24	18 mg.
2.1	S S	S S		19 mg.
2.3	S S	S S		16 mg.
2.5	S S	51 S		
3.1	45 54	24 24		
Original extract control	S S	45 69	24 45	16 mg.
Virulence control cc. of culture		0.000,000,1	0.000,000,01	0.000,000,001
		45 45	45 45	S S

Any bactericidal influence of the copper affecting the protective test was excluded by the following experiment:

Protective Effect of Copper Chloride Solutions.

Each mouse received 0.2 cc. copper solution diluted to 0.5 cc. saline, and the indicated amount of 18 to 24 hour broth culture of pneumococcus Type 1 contained in 0.5 cc. broth.

TABLE 4.

Dose Protective Material	Dose of Culture		0.001
	0.000,01	0.000,1	
0.2 cc. M/2200 copper chloride in saline	S	40	25
	S	88	40
Virulence control dose of culture	0.000,000,1	0.000,000,01	0.000,000,001
	40 40	40 40	S S

This protocol shows that 0.2 cc. of M/2200 copper chloride protects against 1,000 but not against 10,000 fatal doses of pneumococcus. The doses used in the antibody experiments (0.01 and 0.06 cc.) are 1,000,000 and 6,000,000 fatal doses respectively.

The material and mice (of which over 2,000 were used in these studies) were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

103 (2626)

Studies on the purification of antibodies. IV. The removal of extraneous material from antipneumococcus extracts at an approximately neutral reaction.

By R. OTTENBERG and F. A. STENBUCK.

[From the Pathological Laboratory, Mount Sinai Hospital, New York City.]

Experiments with precipitates and supernatant fluids, obtained by bringing antibody-containing extracts of sensitized bacteria to a known range of hydrogen ion concentrations, revealed another method of purifying such antibody solutions of extraneous material, which method is different from that described in the preceding paper. It was found that at hydrogen ion concentrations between pH 7.0 and 7.6, that is, well to the alkaline side of the point of precipitation of antibody itself (about pH 4.0), pneumococcus protective antibody remained in solution, but a large amount of indifferent material, containing considerable nitrogen, was removed from solution. It was possible to remove a fraction of this indifferent material before adding copper chloride; and then, after addition of copper chloride, to remove a further fraction, as illustrated in the following protocol.

Two and a half liters pneumococcus antibody solution were brought to pH 7.0 by the addition of hydrochloric acid. A precipitate formed. This was allowed to stand in the ice box over night, and centrifuged. The supernatant was designated "M-20 C." Trial precipitations of 10 cc. portions of this supernatant by addition of copper chloride indicated complete recovery of the

antibody in the supernatant fluid, after removal of the precipitate formed at pH 7.2, with the copper chloride in final concentration of M/2100. Two liters of "M-20 C." were then treated with 100 cc. of M/100 copper chloride and brought to pH 7.2. A pale green precipitate formed. It was allowed to stand over night in the ice box and then centrifuged. The original material, "isoelectric supernatant" (*i. e.*, supernatant after removal of the first precipitate) and "copper supernatant" were tested on mice. Each mouse received 0.5 cc. of a 1/200 dilution of 18-24 hour pneumococcus Type 1 culture, (*i. e.*, 0.0025 cc. of culture) and 0.5 cc. of the protective material diluted as indicated.

Comparison of original antibody solution and "isoelectric supernatant".

Material	Dilution of Material		Nitrogen per 100 cc.	
	0	1:10		
Original antibody extract	S	48	12.1 mg.	
	S	64		
Supernatant of pH 7.0 isoelectric precipitation	S	48	8.2 mg.	
	S	S		
Virulence control dose of culture	0.000,000,01	0.000,000,005	0.000,000,0025	0.000,000,00125
	48	48	48	S

This shows that the antibody was all recovered in the supernatant.

Comparison of "Isoelectric Supernatant" with Supernatant after Further Precipitation with Copper Chloride.

Material	Dilution of Material		Nitrogen per 100 cc.	
	0	1:10		
Supernatant of isoelectric ppn. at pH 7.0.	72	24	8.2 mg.	
	S	S		
Supernatant of above after copper precipitation.	S	92	3.0 mg.	
	S	S		
Virulence control dose of culture	0.000,000,01	0.000,000,005	0.000,000,0025	0.000,000,00125
	24	48	41	S

This shows that the antibody is still in the supernatant after copper precipitation at 7.2, although this supernatant contains only one-fourth of the nitrogenous matter of the original material.

The removal of indifferent material is easy to bring about, and occurs with much greater regularity than the precipitation of the antibody itself at a more acid point as described in the preceding paper. It would therefore seem to offer a more practical method for partial purification of the antibody.

The material and mice used in these studies were generously furnished by the H. K. Mulford Co., Glenolden, Pa., through the courtesy of Dr. F. M. Huntoon.

104 (2627)

Observations on the extra-cardiac circulation.*

By C. S. DANZER.

[*From the Physiological Laboratory and the Medical Clinic of the Johns Hopkins University, Baltimore, Md.*]

Experiment (I)

If the bulbus arteriosus of the frog is ligated, the heart gradually dilates. In about ten minutes the heart has more than doubled its size. (Fig. 1.) The heart muscle appears more cyanotic. If at the same time the peripheral circulation in the frog's web is observed microscopically, it is seen that the blood keeps moving in the capillaries for 5-10 minutes after aortic ligation. While corpuscular flow is slower, it simulates the normal as far as direction is concerned, going from the arteries to capillaries, then to the veins.

Thus the enlargement of the heart after aortic ligation is associated with the movement of blood from the large arteries to the capillaries, and from these back to the heart. It appears as though co-ordinated contractions of the vessels themselves were capable of circulating the blood along its usual course. This vascular mechanism comes into play when the cardiac output is prevented by ligation of the aorta.

If now the vessels of a second frog are injured quite another reaction takes place following the ligation of the aorta. In order to produce a profound vascular injury, the spinal cord of the animal was destroyed by pithing. This, as is known, has a tre-

* This work was completed in 1919.

FIG. 1.

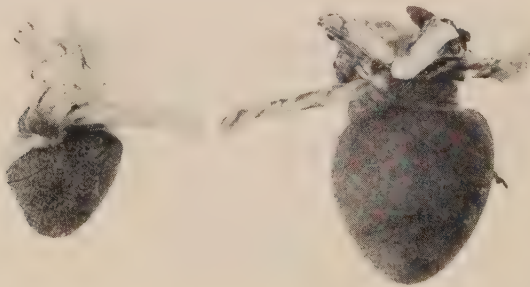


Heart before ligation of Bulbus Arteriosus.

Heart after ligation.

mendous "shock effect." One of the evidences of this is the cessation of spontaneous arterial contractions in the frog.^{1, 2} To further damage the vessels chloroform was applied to the skin of the animal. This caused a marked dilatation of the capillaries, but the corpuscular velocity before aortic ligation did not differ materially from the normal. Now the aorta was ligated. Almost at once the blood-flow in the capillaries stopped, the corpuscles became stagnant. There was now no sign of cardiac enlargement, when the functional integrity of the vessels was impaired (Fig. 2). Hence in the animal with damaged vessels there was neither a peripheral circulation nor an enlargement of the heart after the aorta was clamped. These two effects must therefore be ascribed to the proper functioning of the vessels.

FIG. 2.



¹ Nussbaum, *Pflüger's Archiv.*, 1875, x, 375.

² Huzinga, *Pflüger's Archiv.*, 1875, xi, 207.

To further study the reaction of the peripheral vessels when an artery was ligated centrally the following experiments were performed.

Experiment (II)

The aorta of a cat was clamped, and the time elapsing between this procedure and the stagnation of the capillary circulation in the ear was measured. The corpuscular flow lasted 35 seconds in one capillary, 50 seconds in another. The clamp was now released, and the circulation became reestablished. The aorta was again clamped. This time the corpuscular flow in one capillary lasted for six minutes after arterial compression.

Experiment (III)

A frog's leg was ligated above the tibio-tarsal joint, and quickly amputated just above the ligature. The circulation in the web of the foot was studied microscopically. The corpuscular flow lasted 2-3 minutes after the vessels were tied, in some experiments even longer. An interesting phenomenon observed in this experiment was the intermittency of the corpuscular movement. At times the corpuscles oscillated back and forth. There was a striking similarity between these and the corpuscular oscillations in the capillaries of man when pressure measurements were made with the micro-capillary-tonometer.³

Experiment (IV)

A ligature was firmly tied around the middle of a frog's thigh, and the web circulation studied microscopically. The periodic to-and-fro movement of the corpuscles was again observed. This lasted as long as the period of the observation, thirty minutes.

The successive phases of corpuscular movement were as follows:

Art. to Vein—	3 min.
Vein to Art.—	18 sec.
Art. to Vein—	16 sec.
Vein to Art.—	15 sec.

Experiment (V)

An incision was made into the skin of the frog's thigh, and a subcutaneous artery was selected. This vessel divided into fine capillary branches which were distributed on the under surface

³ Danzer and Hooker, *Am. J. Physiol.*, 1920, lii, 136.

of the skin. A skin flap was then made, and the circulation of the vessels was observed under the microscope. For five minutes after the main artery was ligated the corpuscles kept slowly moving in a normal direction in the capillaries. The movement, however, was not steady but oscillatory in character.

Experiment (VI)

A Riva-Rocci blood pressure cuff was placed around the arm, and the capillaries of the finger of the corresponding hand were viewed microscopically. The cuff was inflated to a pressure of 300 mm. Hg. The interval between the compression of the artery and the cessation of the capillary circulation was measured with a stop watch. The direction and duration of the subsequent movements of the corpuscles were also studied. The results are tabulated below.

	Art.→Vein	Stasis	Vein→Art.	Stasis	V→A	Stasis
Miss C.	15 sec.	30 sec.	75 sec.	20 sec.
D. R. H.	10 sec.	00 sec.	30 sec.
	20 sec.	---	---
Miss C.	37 sec.	10	75	43(?)
C. S. D.	27 sec.	11 sec.	47 sec.	13	70	55

First the arterial
then venous limb of
the capillary empties.

It is apparent that after the cuff has been inflated up to supra-systolic pressure, the corpuscles still keep moving in their normal direction—from artery to vein—for a period varying from 10 to 37 seconds in normal subjects. In pathological cases, or where the arm has been compressed for some time, this phase becomes much prolonged. It may last 100 or more seconds under such conditions. When this prolongation occurs the flow is not a continuous one, but is separated by short intervals into several parts. In the following few minutes the corpuscles become stagnant, then reverse their course, going from vein to artery, then stagnate and reverse their course once more.

The vascular reactions in man following brachial compression probably consist of a slow peristaltic contraction wave followed by intermittent periods of relaxation of the arterial vessels distal to the cuff. If the veins were not simultaneously obstructed, by the latter, this peripheral vascular motor mechanism might drive the blood in the direction of the heart, just as in the aortic ligation experiment of the frog.

Experiment (VII)

To bring out more clearly the differences in reaction between normal and injured vessels the following experiment was done:

A frog was anesthetized with ether. The sciatic nerve on the left side was cut, and chloroform was applied to the web of the corresponding foot. The right lower extremity was left intact. The webs of both feet were separately fixed on two boards and viewed with two microscopes. By means of a Leitz "Comparison Ocular," the visual fields of both were observed simultaneously.

The bulbus arteriosus was ligated and the circulation in both webs observed.

In the right web: (normal)

In the left web: (sciatic section and capillary relaxation)

The corpuscular after-flow lasted three and half minutes. This represents the vascular reaction in the normal extremity.

The blood corpuscles stopped moving almost immediately after the aorta was ligated. This represents the reaction of severely injured vessels.

This experiment proves that, when the vasomotor function is eliminated and the capillaries are dilated as in the left foot of the frog, aortic ligation is followed by immediate cessation of corpuscular flow in the vascular areas supplied by these vessels.

Injury to the vasomotor system or the local application of vasodilators alone does not produce a very striking effect. The combination of the two procedures, however, paralyses the peripheral vascular mechanism and prevents the filling and enlargement of the heart after aortic ligation.

This and the previous experiments have established for us the following working hypothesis:

(1) The size of the heart after ligation of the aorta is an index of the integrity and function of the peripheral vascular mechanism. The latter is elicited by arterial ligation.

(2) The peripheral vascular mechanism may be inhibited by the combined action of local vasodilator substances (chloroform, etc.) plus the destructive effect on the vasomotors by nerve section or spinal cord pithing.

105 (2628)

Blood calcium in relation to sex in pigeons.

By OSCAR RIDDLE and HANNAH E. HONEYWELL.

[From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, New York.]

Some years ago Reach¹ made comparisons of the amount of calcium recoverable from ashing the entire bodies of male and female white mice. His examination included fair numbers of normal males and females, and of other males and females which had been castrated 42-108 days. He obtained higher percentages of CaO from both groups of females (1.283 normal; 1.275 castrated) and lower percentages from the two groups of males (1.180 and 1.005). Regarding the utilization of calcium, Reach drew the conclusion that "we have here a *secondary sexual character*—the females of these animals are richer in calcium than the males." Lawrence and Riddle² later showed that there is more phosphorus in the *blood serum* of female than of male fowls; and that still larger amounts of phosphorus are found in the blood of the laying hens than of non-laying hens.

In view of the known relationships of the calcium and phosphorus of the blood, both of the preceding studies suggest that the sexes—at least in birds—may show differences in their calcium metabolism. Because of its bearing on the problem of sexuality in pigeons the present study was undertaken. Present methods of blood calcium determination probably warrant the assumption that a sufficient number of determinations made on normal carefully controlled birds will reveal differences, if any very notable sexual differences exist. This report, however, does not represent an adequate study; it deals with the data obtained in a first attack on the problem.

The Kramer and Tisdall method was used. Our determinations therefore are for calcium plus appreciable but unknown amounts of magnesium. Along with each serum sample a check determination was made on oxalate solution of known strength. Adult birds of three kinds were taken for study; most of the pairs

¹ Reach, F., *Biochem. Zeitschr.*, 1912, xvii, 59.

² Lawrence, J. V., and Riddle, O., *Am. J. Physiol.*, 1916, xli, 430.

(except "family hybrids") were in a more or less active state of reproduction. The age and the obvious reproductive history of all birds were exactly known. All birds—at least the male and female of each pair studied—had been subject to closely similar conditions of diet, light, confinement and parentage. Some of the birds proved at autopsy to be infested with intestinal worms (*Ascaridia*) or were otherwise not entirely healthy. The members of a pair were usually not of identical age. An attempt was made—only partially successful—to obtain two determinations from each bird at intervals of about 10 days. It was thus necessary to draw the first sample from a wing-vein, and this often failed to yield 2.0 cc. serum. The second sample was taken from the decapitated bird. No anticoagulant was used, and in a few cases even the serum coagulated on standing and prevented calcium estimation. It now seems probable that the drawing of samples from the wing-vein was unwise since failure to autopsy the bird immediately led to occasional error in judging its reproductive state.

TABLE I.
Calcium in blood serum of male and female pigeons.

Kind and sex of pigeon	First determination		Second determination		Range of values found	
	No. of birds	Calcium (mg. per 100 cc.)	No. of birds	Calcium (mg. per 100 cc.)	No.	Values
Common pigeons	♂ 7	9.81	9	10.00	16	9.1-10.9
	♀ 6	12.24	8	11.27	14	8.9-18.9
Ring doves	♂ 2	9.05	5	10.58	7	8.2-12.0
	♀ 4	12.41	7	12.78	11	9.5-17.3
Family* hybrids (♂)	8	10.86	12	10.43	20	8.7-12.5
Other determinations made on samples of less than 1 cc. serum.						
Common pigeons	♂ 3	12.83			3	9.0-15.0
	♀ 2	15.60			2	12.0-19.2
Ring doves	♂ 3	10.35			3	9.4-11.6
	♀ 3	15.81			3	12.4-18.8
Family* hybrids (♂)	5	11.82			5	8.5-16.6

*From a cross of ♂ common pigeon x ♀ ring dove. All these hybrids were producing sperm, but some of them were probably zygotic females; two of the 13 had left oviducts.

The results are recorded in Table 1. If individual, instead of group or average, determinations were tabulated they would show a greater diversity than we have been able to indicate in the summary given here. The two determinations for the same bird—even in the case of the males—sometimes completely fail to check, and this divergence is pronounced in the case of females. The determinations made on samples of less than 1.0 cc. serum are known to be untrustworthy and are usually discarded. Averages of these are given in order that the whole of our data may be presented; they show both higher values and a wider variation than were obtained from adequate samples.

Altogether the table offers a comparison of values obtained from six groups of males with six groups of females. Considered as groups, the males show the lower, females the higher, calcium-magnesium values in every case. If one attempts to group the individual data on the basis of health, age, or body weight, no significant correlation is obtained. Only on the basis of sex have we been able to arrange a consistent grouping of the values found.

The "family hybrids" require further mention. Common pigeons (*Columba*) and ring doves (*Streptopelia*) belong to different zoological families, and when crossed their offspring are males only. The 13 birds of this origin used here resulted from such a cross and were of the same fraternity. Other data to be published elsewhere supply evidence that, although they all produced sperm, some of them were probably zygotic (chromosomal) females. It will be noted that these hybrids, considered as a group, had a blood calcium value intermediate to the values found for the normal males and females of the two parental species. It can not be said that the particular individuals otherwise suspected of being zygotic females showed the higher calcium values. The two most marked cases (presence of oviducts) of relative "femininity" among this group, however, do give a higher average ($4=11.16$; $16=10.45$), and one of these two showed the highest value obtained for any of the 13 birds.

In connection with the studies earlier mentioned in this paper, it should further be noted that in the femur and humerus of rats, earlier thyroparathyroidectomized at 100 days, Hammett³ found the amount of calcium unchanged in the males, but somewhat

³ Hammett, F. S., *J. Biol. Chem.*, 1923, lvii, 285.

less than normal in the females. This case again suggests some difference in the calcium metabolism of the two sexes.

It remains to point out that the data here reported may be unduly influenced by the inclusion of females actively producing eggs, and thus with specially active calcium glands in the oviducts of some of the females. Data since obtained, and to be reported later by Riddle and Reinhart, indicate that this is the case. We here report no data on juvenile birds, nor on virgin females. In the present data, and on the type of adult pigeons used by us, a difference in the calcium metabolism of the sexes is found; the various groups of females show notably higher amounts of calcium in the blood.

106 (2629)

A spasm-inciting substance in the sputum during asthmatic attacks.

By JOSEPH HARKAVY. (Introduced by R. Ottenberg).

[*From the Institute of Pharmacology, University of Leiden—Prof. Strom Van Leeuwen, Director.*]

Studies were carried out on eight cases of bronchial asthma whose ages varied between 25 and 56. Six of these cases had been found to be sensitive to one or more foreign proteins. Special care was taken to collect the sputa of every patient at the height of the attack, for it was thought that that would be the most favorable time to obtain the spasm-producing substance, if any such was present. The sputa thus obtained were extracted in alcohol, with the exception of two instances in which studies were made in the fresh state after being dissolved in Tyrode solution. The precipitate obtained with alcohol was also dissolved in Tyrode solution before using. The material obtained in this manner was tested on isolated strips of smooth muscle from cat's intestine, suspended in Tyrode solution. Prior to the actual experiment the constant contractility of the muscle to pilocarpine was first established.

As a result of these investigations it was found that the sputum of the asthmatics studied contained a substance which stimulated smooth muscle to contract. In most of our cases the contraction

was prompt, spasmodic and sustained in character. In others it was slower but equally sustained for periods of five to seven minutes. In four cases where the sputum was studied in the interval of attacks, *i. e.*, before and after the attacks, no contraction of smooth muscle was obtained—the kymographic registration showing a flat curve.

Control sputa prepared in a similar manner to the above from five cases of pulmonary tuberculosis, three of bronchiectasis, and five normal individuals gave absolutely negative results.

Further investigations are in progress to ascertain the nature of the substance and its significance.

107 (2630)

Proteins of the cotton seed.

By D. BREESE JONES and FRANK A. CSONKA.

[*From the Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington, D. C.*]

By extracting finely ground cottonseed kernels (hull-free) with benzene (C_6H_6) nearly all of the fatty and resinous substances and much of the coloring material is eliminated. For this purpose benzene is far superior to ether. Such a thorough removal of the above substances from the flour greatly facilitates a satisfactory subsequent extraction of the proteins by different solvents. The high percentage of nitrogen extracted by sodium chloride solution (Table I) is doubtless due to the method of preparation of the flour.

TABLE I.

	Percent of total N.
Salt soluble protein N.....	76.6
Alkali soluble protein N.....	8.2
Extractable non-protein N	10.1
Residual N (by difference)	5.1

We were able to separate from the salt extract two globulins. Of these one can be precipitated directly at 0.4 to 0.5 of satura-

tion with ammonium sulfate. The other flocculates at a saturation of 0.7 to 0.8, but only after it is diluted with water so that the ratio of the final volume to the weight of the original cotton-seed flour extracted is as 50:1.

A fraction having a relatively low nitrogen content, but containing a constant and very high ash percentage was obtained from the salt extract by coagulation; the composition of this preparation points to a nucleic acid. The examination of this product is under way and the results will be published later.

The globulins were prepared by reprecipitation with ammonium sulfate followed by dialysis. The identification of glutelin and a nucleo protein present in the meal, and also the chemical analyses and determination of certain physical constants of the different protein fractions are under way.

108 (2631)

The development of antirachitic potency in phytosterol and cholesterol following irradiation.

By ALFRED F. HESS, MILDRED WEINSTOCK and F. D. HELMAN.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In a previous communication¹ it has been shown that when vegetable oils are activated by means of ultra-violet irradiation, the antirachitic factor is confined to the non-saponifiable fraction. Further investigation has demonstrated that phytosterol obtained from these oils, although unable to protect against rickets, can in the same way be endowed with antirachitic potency. In these tests the phytosterol was suspended in water, and 0.25 cc. of a 1 percent suspension was fed daily to each rat. Similar experiments were carried out with cholesterol, of which 0.25 cc. or 0.1 cc. was fed. It was found that cholesterol was also able to prevent rickets following irradiation when fed to rats receiving the low phosphorus rickets-producing diet. Spectrograms showed that following irradiation the cholesterol was

¹ Hess, A. F., Weinstock, M., and Helman, F. D., *Proc. Soc. Exp. Biol. and Med.*, 1924, **xxii**, 76.

altered so as to transmit a greater intensity and a wider range of the shorter ultra-violet radiations. Lanolin which had been irradiated with the mercury vapor lamp to the same degree—for one-half hour at a distance of one foot—was found to have acquired much less antirachitic potency.

109 (2632)

Crystals of vitamin B from the Mung bean.

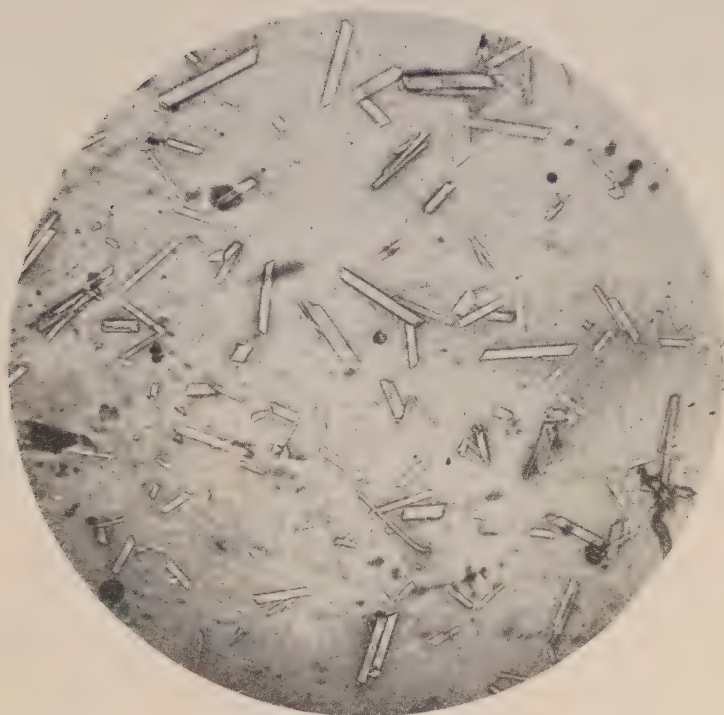
By H. H. M. BOWMAN and MARTIN A. YEE.

(Introduced by V. K. La Mer).

[From the University of the City of Toledo, Toledo, Ohio.]

The source of the vitamin is a Chinese bean, the Mung bean, ground dry into a meal. The method of extraction is a modification of Suzuki's (1912). The meal is boiled with methyl alcohol (80 percent) plus 2 percent hydrochloric acid, and extracted three times in a reflux condenser. The filtrates are added together and evaporated under reduced pressure. Precipitates are filtered off as evaporation proceeds. The evaporation is continued to the removal (complete) of alcohol. All fats, organic acids, etc., are removed from the aqueous solution with ether. The solution is then acidified with sulfuric acid and treated with phospho-tungstic acid to complete precipitation, and filtered. The precipitate is decomposed with a solution of acetone made alkaline with barium-hydroxide, the resulting precipitate being filtered off. The excess of barium-hydroxide is removed with sulfuric acid, and excess of sulfuric acid is removed with lead acetate, and excess of lead removed by hydrogen sulfide. The resulting solution is then evaporated. When evaporation has reached approximately 3 percent of the initial quantity of liquid used in the extraction, needle-shaped, colorless crystals begin to appear. When evaporation is carried to dryness the crystals coalesce as brownish masses. This mass may be recrystallized as white crystals from 80 percent ethyl alcohol.

The melting point of these crystals is 320 degrees Centigrade, and they turn brown at 245 degrees Centigrade. The identity of these crystals as uric acid crystals could not be established by



Microphotograph of crystals of Vitamin B isolated from *Phaseolus aureus*.



FIG. 1. Pigeon in which acute beri-beri was induced, showing typical neck retraction.



FIG. 2. The same bird as in Fig. 1, one hour after receiving a dose of 2 mg. of Vitamin B crystals, solution being injected in the pectoral muscle.

appropriate tests, nor as amino acids, nor as protein with proper tests for these substances. With bromine water they give a yellow precipitate, and a blue color with the Folin-Macallum Reagent.

Polyneuritis was induced in pigeons by putting them on a diet of polished rice and water for about two weeks. In birds suffering with the acute symptoms (*i. e.*, retracted neck muscles and paralyzed legs and drooping wings), these acute symptoms were relieved by injecting minute doses of three milligrams of the crystals dissolved in water and dilute alcohol, into the pectoral muscles. Within an hour these pigeons were able to stand erect and showed normal movement of the head. This was repeated with four adult pigeons each weighing on an average about 345 grams. They were placed on the restricted diet and weighed each day and the weights recorded from which, later, curves were made. Two of these pigeons were given doses of the solution of three milligrams of the vitamin crystals at intervals of three days. The curves, while showing a steady decline in weight, show a sharp upward trend after each injection of vitamin into the pectoral muscle due to the renewal of the appetite and consequent eating of more polished rice. The other pigeons kept on declining until the end of the experiment. Charts were kept of each pigeon individually.

Growth experiments were also made with guinea pigs and the crystals, but the development of scurvy complicated conditions unless special diets to prevent scurvy were used. Pigeons on the whole are much more satisfactory subjects in studying Vitamin B or the anti-beri-beri accessory.

110 (2633)

The use of chloretone as an anesthetic for paramecium.

By WILLIAM H. COLE and EUGENE RICHMOND.

[*From the Biological Laboratory of Clark University,
Worcester, Mass.*]

The described methods for quieting paramecia by using formaldehyde, quince seed jelly, cotton fibers, etc., are by no means satisfactory, if observations are to extend over more than a few

minutes. They are of no use at all for observing a single animal, or a particular group of animals, over a period of several hours or days. Experiments have been made with chloretone* (trichlortertiarybutyl alcohol), and have shown it to be especially valuable for quieting paramecia for long periods of time, up to 8 days, with no undesirable effects.

The paramecia used were extracted from wild cultures, and grown in hay infusion of known constant concentration. New cultures were started from time to time by adding a pipette full of the old to fresh infusions. These cultures contained a few of the smaller rotifers, many of the smaller ciliates, and a preponderance of *Paramecium caudatum*, a mixed population which seemed to be favorable for their growth and reproduction. It was found that a solution containing about 0.06 percent chloretone by weight would anesthetize paramecia in a few minutes, and keep them anesthetized for varying periods of time. The animals would recover if placed in fresh culture fluid previous to cytolysis.

The technique consisted of placing 1 drop of culture fluid, densely populated with paramecia, in a shallow glass chamber on a slide, and then adding 1 drop of 0.12 percent chloretone solution. After measuring the drops of the two fluids from their respective pipettes it was found that such a mixture contained 0.056 percent chloretone. In all subsequent tests the same two pipettes were used. The size of the drops can be adjusted so as to yield just enough fluid to fill the chamber. The latter was then sealed with a cover glass using a mixture of bee's wax and white vaseline, the consistency of which just allowed of its easy spreading at 20° C. (approximately 1 part of wax to 3 parts of vaseline). Gentle heat applied to the cover insured an air-tight seal. Vaseline alone did not give good results, since the animals always recovered within a few hours (from 2 to 24), a fact so far unexplained satisfactorily. It was noted that a few individuals are sometimes killed immediately upon the addition of the chloretone, due probably to the fact that they come in contact with the chloretone before it has been diluted with the culture fluid.

After a maximum of 1 hour the majority of the paramecia come to rest and remain so for 24 hours. During this period

* After the completion of this abstract a casual reference to the use of chloretone for "partially stupefying" paramecium by Jennings was found. Jennings, H. S., *J. Comp. Neurol. and Psychol.*, 1904, xiv, 442.

there is no movement of the animal as a whole, although the cilia in the oral groove, the undulatory membrane, the contractile and food vacuoles continue to show their usual movements. In any large group of the animals there will be several different positions of rest, so that observations on special structures, such as the gullet, contractile vacuoles, "anus," etc., or on protoplasmic streaming, become easy to make. Only rarely does a paramecium become deformed or show cytolysis at the concentration of 0.056 percent before the end of the second day.

The length of the period of anesthesia varied widely in the different chambers. In general a concentration of 0.056 percent was effective for 2 days, many of the animals then resuming locomotion for from 3 to 6 days. After recovery, all of the animals died within 24 hours. In one case the paramecia remained quiet for 8 days, recovering on the ninth day, and dying during the tenth day. Other chambers showed quiet animals up to 4, 5 and 6 days, although some of the paramecia in each chamber were active. The reason for the variation in the recovery process in the different experiments is a subject of further investigation.

In several cases there was noted an increase in the numbers of the smaller ciliates, but in only one case did the number of the paramecia increase slightly. In other words the method is not favorable for the reproduction of paramecium, although it may be for other forms. It is interesting to note that none of the smaller ciliates were ever anesthetized at the concentration of 0.056 percent.

By increasing the concentration to about 0.066 percent and using the ordinary slide and cover, the method becomes applicable to elementary class work, and has given excellent results in this laboratory. The paramecia become quiet within a maximum of 10 minutes and a large number will remain so for several hours without showing any deformity or interruption of the functions mentioned above, provided evaporation is compensated by adding 0.066 percent chloretone solution at the edge of the cover whenever necessary. A few animals will cytolysed and die within the first hour, and the whole population will be dead after a maximum of 24 hours.

Further experiments are in progress on the specific functions of paramecium by the chloretone method, and also on the application of the method to other protozoa.

111 (2634)

Extractives of muscle: a new iminazol phosphorus compound.

By WILSON D. LANGLEY. (Introduced by D. Wright Wilson).

[*From the Department of Physiological Chemistry, Medical School, University of Pennsylvania, Philadelphia, Pa.*]

For the separation of the mixture of organic substances which occurs in muscle, the Kossel-Kutscher¹ procedure has been found to be inadequate. It causes decomposition of certain substances present, and does not satisfactorily separate the resultant decomposition products. A different method for the isolation of the extractives of muscle is therefore being developed. Before the new method can be extended, it becomes necessary to identify some of the products obtained.

One of these is a copper salt of an unstable phosphoric acid compound, of which the empirical formula obtained is $C_{14}H_{21}O_{11}N_4P \cdot 4H_2O$. The copper salt has been obtained nearly pure by repeated precipitation from a concentrated water solution by alcohol. It is very hygroscopic, and loses 4 molecules of water when heated at 120°.

The substance gives a strong color reaction for the iminazol ring, but no other color tests have been obtained. It does not reduce Benedict's solution, nor does it give a positive reaction for pentose. The phosphoric acid may be precipitated in the cold by alkaline magnesia mixture, although it was not removed by treatment of the compound with barium hydroxide in the process of its isolation, or by copper oxide, in the preparation of the copper salt. The evidence for the phosphoric acid being present other than as a salt, is considered, however, to be incomplete.

If, from the iminazol color reaction, the presence of carnosine in the molecule is assumed, the organic residue, other than carnosine, would then have the composition $C_5H_6O_5$. It would seem that this residue has come from some carbohydrate decomposition product.

This iminazol phosphorus compound therefore is a highly unstable substance, which should correlate the chemistry of carnosine and of a carbohydrate, and possible also of loosely bound phosphoric acid in muscle.

¹ Isolierung von Basen aus den Extrakten der Muskeln. D. Ackermann. Abderhalden's Handbuch der biochem. Arbeitsmethoden, 2, 1002.

112 (2635)

The absorption and excretion of carbon tetrachloride in animals and in man.

By HERBERT S. WELLS. (Introduced by Paul D. Lamson).

[From the Department of Pharmacology, Johns Hopkins Medical School, Baltimore, Md.]

To date, the study of the toxicity of the anthelmintic dose of carbon tetrachloride has been confined to clinical observations, and to pharmacological and pathological findings. These researches have indicated that the damage to the host following the use of this drug is definitely dependent on its absorption from the intestinal tract.

Quantitative chemical methods for the study of absorption and excretion of carbon tetrachloride have been worked out in this laboratory.* The details of the methods together with a full report of the results of their application will be published elsewhere at an early date.

By the use of our methods we have been able to throw considerable light on the absorption and subsequent excretion of the anthelmintic dose of the drug in man as well as in animals. In dogs, absorption has been studied following injection of 3 cc. of the drug (the accepted therapeutic dose for dogs and for man) into loops of intestine prepared by tying off duodenum and colon under general anesthesia. The dogs were killed at intervals ranging from one to thirty hours, and the amount of carbon tetrachloride remaining in the gut determined. In this way it was shown that the whole dose is absorbed in 24 to 30 hours. The rate of absorption remains practically constant from beginning to end, though it is somewhat more rapid at first. Absorption thus determined for eighteen dogs gives data for the construction of a remarkably smooth composite curve of absorption. When 50 percent alcohol is added, up to 100 cc., there is no general tendency to increased absorption. When 97 percent alcohol is added, on the other hand, absorption is markedly accelerated during the first few hours. The addition of 10 cc. of saturated magnesium sulphate solution slightly decreases the amount absorbed.

* Aided by grant from the International Health Board. Grant directed by Dr. P. D. Lamson.

When the expired air of dogs is passed, for absorption, through activated charcoal, a very high percentage of the carbon tetrachloride absorbed from an intestinal loop is shown to be rapidly excreted by the lungs. In one case 96 percent of the amount lost from the gut was so recovered.

Studies of the expired air following the taking of two small doses of carbon tetrachloride by the author himself (3 cc. and 1.5-2.5 cc. respectively) show that the drug can be much more rapidly absorbed and excreted by man than by dogs. The maximum rate of excretion, which was shown in one instance to occur within 40 minutes of administration of the drug by duodenal tube, coincided with the greatest intensity of subjective sensations of dizziness and of the odor of the substance on the breath. The rate of excretion fell rapidly thereafter, following a smooth curve, which practically reached the baseline in 24 hours. The results of these experiments suggest that the rate of absorption in man depends on the concentration of the drug in the small intestine.

Another possible explanation of the rapid decrease in rate of absorption is that offered by Schultz and Marx.¹ These authors suggest, on the basis of an excellent pathological study of liver lesions produced in dogs, but on what seems to us to be inconclusive evidence, that absorption is most rapid from the duodenum, and that as the drug is carried further down the gut the rapidity of absorption decreases. That this does not seem to be the case in dogs, however, is shown by the results of our experiments, in which the rate of absorption of the drug, following injection into the duodenum, decreases only slightly at first and proceeds at a constant and relatively high rate after the first few hours.

It cannot be denied that the phenomenon of the rapid decrease in rate of absorption which apparently occurs in man might be explained by the hypothesis put forth by Schultz and Marx. Yet our experiments on man indicate that it is not always possible to draw adequate conclusions about absorption in man from the quantitative results of experiments performed on dogs. Consequently, further experiments on man will be needed before it can be settled definitely as to whether the rate of absorption de-

¹ Schultz, E. W., and Marx, Alberta, *Am. J. Trop. Med.*, 1924, iv, 469.

pend on the concentration, as our experiments up to date would lead us to believe.

It is suggested, in the light of the rôle which the concentration seems to play on absorption rate, that should the drug be administered in divided doses, the concentration reaching the tissues would be reduced and the time during which the drug remains in the intestine prolonged. By such a method of administration of the drug, the toxicity might be considerably reduced for selected patients in whom the factor of safety would outweigh that of ease of administration.

The study of the excretion of carbon tetrachloride in the expired air (which presumably occurs almost as rapidly as the drug is absorbed) seems to offer the best avenue of approach to the study of absorption of the drug in man, and of the effect of various substances that tend to accelerate or retard absorption.

113 (2636)

The determination of blood sugar.

By STANLEY R. BENEDICT.

[From the Department of Chemistry, Cornell University Medical College, New York City.]

A method for the determination of blood sugar has been developed which is based upon the use of a more specific copper solution than has hitherto been available. Where the new procedure is employed for normal human blood the results are from fifteen to thirty percent lower than those obtained where the method of Folin and Wu is employed. Details of the procedure will be published in a short time.

114 (2637)

**On the experimental production of lack of carbohydrates, and
on the carbohydrate metabolism of the central nervous system.**

By LEON ASHER and KISHI TAKAHASHI. (Introduced by
Holmes C. Jackson).

[*From the Physiological Institution of the University of Berne,
Hallerianum, Switzerland.*]

To gain further insight into the genesis of the hypoglycaemic symptoms and other problems of carbohydrate metabolism it appeared advisable to dispose of various methods of depriving the organism of its stores of carbohydrates. In various former publications from the Berne Physiological Institute, mention has been made of methods suitable for this purpose. We have now worked out such a method that one is able experimentally to deprive the organism almost completely of its store of carbohydrates. The method, as applied to rats, consists in the following procedure: The rats are fed for a few days with Witte's pepton, then with thyroid gland preparations, and finally phlorhizin is injected according to Coolen's methods, and the animals are then worked in a treadmill. The treatment with Witte's pepton and with thyroid gland preparations must be carefully regulated according to the condition of the animals; they are especially sensitive to feeding with Witte's pepton. With this method we succeeded in lowering the carbohydrates of the liver 96 per cent, the carbohydrates of the muscle 85 per cent, and the blood sugar to the same low value as in the hypoglycaemic state after insulin.

We were particularly interested in the carbohydrate content of the brain. We found per gram of brain an average amount of 1.23 mg. carbohydrate of which 0.75 mg. is glycogen. This so-called glycogen is the product of analysis according to the method of Pflüger.

As a result of our experimental proceedings the carbohydrates, especially the content of glycogen, had been diminished by something like 90 per cent, the glycogen content of the brain had not diminished more than about 20 per cent. Also after insulin, where we obtained in the case of the liver, the muscles and the blood, the same results as were attained by the method described

above, the glycogen content of the brain was only slightly decreased. The amount of this diminution seemed to depend upon whether or not the animals showed symptoms of convulsions. Therefore we injected strychnine. Like insulin this drug only causes a type of convulsions which is not very marked. Although the decrease in glycogen content of the brain in these cases was more pronounced, the diminution of the carbohydrates of the tissues is at the same time very much less than in the former experiments.

We then turned to rabbits as the effect of insulin upon these animals is far more pronounced. In normal rabbits, on the average, the sum total of the carbohydrates of the brain was 0.79 mg. per gram of brain, of which 0.39 mg. was glycogen. Injections of insulin, when they led to the well known strong convulsions, diminished the glycogen storage of the brain over 80 per cent. While if insulin was given without leading to convulsions, we observed a small but distinct increase of all the carbohydrates of the brain, a fact not without interest, but which is not to be discussed in this preliminary communication. The direction in which our experiments point seems to be clear: Convulsions which are due either wholly or partially to excitations of the central nervous system are capable of diminishing the carbohydrate store of the central nervous system. To prove this, we injected picrotoxin into the rabbit. This produces, by central stimulation, powerful convulsions of a distinctly different type to those occurring after insulin. There is also another difference: The convulsions are accompanied by hyperglycaemia. But what is most important, the glycogen of the brain is decreased 80 per cent, the greatest decrease we ever met with.

Before discussing the bearing of our results, a word has to be said about the chemical nature of the carbohydrates of the brain. As we had employed Pflüger's method of estimation of glycogen we spoke of glycogen in the brain as the precursor of the sugar, which ultimately was estimated. The possibility had to be taken into consideration that instead of glycogen one of the cerebrons served as the source of the sugar. This we actually found. There appeared also to be some physical-chemical difference in the properties of glycogen of the brain prepared by Pflüger's method in comparison with those of glycogen derived from the liver or from the muscle. We therefore tried another method recently

published by Rona and van Eweyck.¹ This procedure does not employ concentrated solutions of caustic potash. We altered it slightly for quantitative purposes, the chief modification being the preliminary treatment with a 3 per cent solution of sodium fluorid, which replaces somewhat in its dissolving capacity the quality of concentrated caustic alkali solutions. The substance prepared by this method from the brains of rabbits gave the characteristic reaction of glycogen, and we therefore believe to have proved the presence of glycogen in the brain. As far as our present studies go, it appears, that not all of that which according to the Pflüger method is estimated as glycogen is in reality glycogen, for the new method gives a smaller amount.

We were able, using the new method, to compare the brain of a patient who died in tetanic convulsions with that of another not suffering from a disease accompanied by a restless state of the muscles. The former brain was devoid of glycogen, while the latter showed approximately the same amount we had found in the brain of rabbits.

The principal result of our studies is the definite proof of a carbohydrate metabolism in the brain, *i. e.*, of a metabolism which allows of carbohydrate storage in the central nervous system. This carbohydrate storage is most resistant, and is, in its resistance, only surpassed by that of the carbohydrates in the heart. The stored carbohydrates of the brain are drawn into metabolism by conditions which favour a state of exaggerated excitability of the central nervous system.

¹ Rona and van Eweyck, *Biochem. Z.*, 1924, *clxix*, 174.

115 (2638)

Studies to determine the biological significance of the vitamins.

By MONTROSE T. BURROWS.

[*From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri.*]

Earlier authors have fully appreciated that conditions other than food and oxygen are necessary for an active growth of cells in the body. This fact is well exemplified in the work of Morgan¹ on the regeneration of the legs of salamanders. Morgan noted that the legs of these animals regenerate as rapidly in starved as in well fed animals. I have sought these other conditions by means of the tissue culture, and find they are a *crowding of the cells* and *stagnation*. Single isolated cells or small groups of cells will not grow in a drop of plasma. For these cells to grow they must be crowded with other cells to form a compact mass of considerable size, and be placed in a small amount of stagnant medium so that the loss of soluble materials from the mass is reduced to a minimum. In the presence of oxygen the cells in such a mass begin to grow after a given latent period.²

In analyzing these factors of cell crowding, stagnation, oxygen and latent period more carefully, I have further found that they signify that growth depends on the accumulation of a certain concentration of an oxydative product of these cells. This product can be readily extracted with salt solution, and when added in a certain concentration to a drop of plasma it will stimulate growth in isolated cells placed in the mixture. In lower concentrations (S_2) it stimulates these cells to migrate and store proteins and fats. Only in certain high concentrations (S_3) can the cells digest these proteins and fats and grow. In all higher concentrations (S_4) it leads to the digestion of the protoplasm of the cells themselves. I have named this substance or substances the *archusia*, the driving substance of the cell.^{3, 4} It corresponds to the heat in the steam engine. It cannot accumulate in any tissue

¹ Morgan, T. H., *Jour. Exp. Zool.*, 1906, iii, 457.

² Burrows, M. T., *Trans. Cong. Am. Phys. and Surgeons*, 1913, ix, 77.

³ Burrows, M. T., *South. Med. Jour.*, 1924, xvii, 233.

⁴ Burrows, M. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, xxi, 94.

having a rich and active blood supply unless the blood has become saturated with it from other sources.

Through the careful extraction of the various tissues of the body it has been found that the growth rate of these tissues corresponds to the amount of *archusia* contained within them. This concentration of the *archusia*, it being a normal product of the oxydative reaction of the cells, can be increased by crowding the cells and reducing the circulation to them, or it may be maintained from outside sources. Cancer I have shown to be nothing more than the result of a primary crowding of the cells together and a relative reduction in their blood supply. As the crowding and the stagnation reaches certain proportions, the mass acquires the property of independent growth. This mass continues to reproduce itself in that it preys upon and destroys surrounding tissues and blood vessels.⁵

The active growth in the bone marrow, nails, sex glands and in wounds is maintained by the same conditions of stagnation of circulation and cell crowding.⁵ These factors are only less in degree than in cancer. In development the greatest growth is in early life. This growth wanes with the development of the blood vascular system and an active circulation. The embryonic tissue of the 5 day old chick-embryo differs from cancer only in that the number of cells per unit capillary area is greater in cancer or the blood supply is less. To transform an embryonic or adult fragment to cancer, it is necessary to stimulate a growth of cells outside the blood vessels (to increase the cells per unit capillary area), or reduce the number of capillaries in the fragment without disturbing the cells. Unchanged embryonic fragments when transplanted into a host form benign tumors; when the cells in them are increased proportionally over the blood vessels they become malignant tumors when transplanted to a host.⁶

By these observations and others on coal tar,^{7, 5} etc., we have found the nature of the cancerous organization and the manner by which it is brought into existence. How the normal functioning organism is developed, and how it maintains its organization remained for solution. In comparing the amount of stagnation and cell crowding necessary for a growth of body cells, it was

⁵ Burrows, M. T., *J. Med. Research*, 1924, xlv, 615.

⁶ Burrows, M. T., *J. Mo. State Med. Assoc.*, 1923, xx, 145.

⁷ Jorstad, L. H., *Proc. Soc. Exp. Biol. and Med.*, 1923, xxi, 67.

noticed that most tissues, even those of the foetus and the young child, are too richly supplied with blood for growth to intervene, unless their blood be supplied with growth stimulus from other sources. We have sought a source for this stimulus in the glands of internal secretion and in the food. Johnston and I⁸ have studied by a new method the action of the ovary in this capacity. We have found that the Allen, Doisy hormone stimulates an active digestion of fat and an active growth of the subcutaneous connective tissue cells. Other authors have noted its action on the uterus, breasts and other sexual organs.

Edwin Smith in his work on the production of tumors by the *B. tumefaciens* showed that this organism stimulates an active growth of cells in the plant. Recently Blumenthal, Auler and Meyer⁹ have isolated a similar organism from human cancers, and reproduced cancers in plants and animals. The action of this organism on the animal cells is quite identical to the extracts of any actively growing tissue from the animal as noted above. It became of interest to see what might be the action of this organism when fed to animals. Jorstad and I have fed two day old cultures of this organism as well as similar cultures of the *B. campestris*, an organism which produces a primary stimulation and then a destruction of plant cells. Chamber¹⁰ while working here in the laboratory showed that the *B. campestris* destroys the plant cells through its ability to break up starch. This ability to split starches does not develop early but late in the cultural life of the organism.

For controls animals were fed:

Potato Starch	80	grams
Egg Albumin	50	"
McCullum Salt Mixture	10	"
Autolyzed Yeast (Vegex ¹¹)	10	"
Butter	30	"

In the experiment 20 cc. of a 2 day old culture of the organism in a simple potato decoction were substituted for the yeast. In other experiments the butter was replaced by crisco to determine if the organism replaces vitamin B (yeast), vitamin A, or both.

In the accompanying curve 1, the results of one of these experiments are given. Both bacteria act in a diet with butter as active and normal growth stimuli. Growth fails when the butter

⁸ Presented 1924, *Am. Soc. Exp. Path.*, Washington, D. C.

⁹ Blumenthal, Auler and Meyer, *Zeitsch. fur Krebsforsch.*, 1924, **xxi**, 387.

¹⁰ Article in Press.

is left out of the diet. These bacteria replace vitamin B, but contain no noticeable amount of vitamin A.¹¹

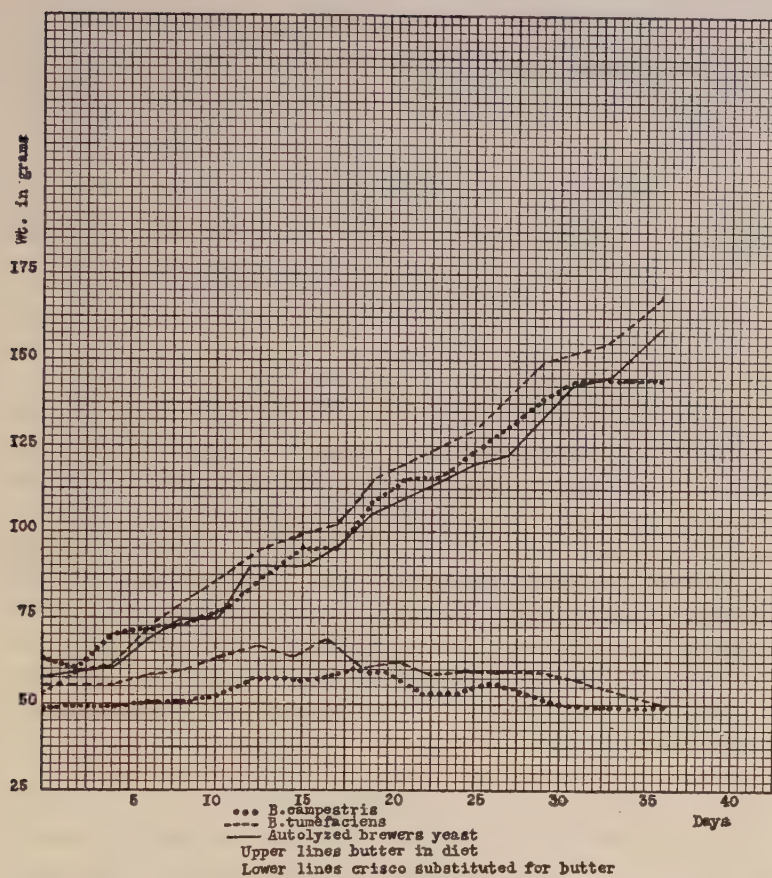
As is noticed therefore, these organisms when placed outside the vessels or into the tissue, stimulate a densely cellular and non-vascular or cancerous organization. When transmitted inside the blood vessels where they act on the endothelial cells first they stimulate the growth of a normal vascular and functioning tissue.

In other papers on muscular contraction,¹² I have shown that function is the result of a polarization of the cell. This polarization is induced by the growth of blood vessels and the rate of circulation. This increased blood supply inhibits growth and utilizes the same energy for function. It is possible, as the above experiments indicate, that the body survives in that it has certain internally secreting glands adapted to liberate stimuli and in preying on lower growing and non-functioning forms. If this be true the higher function in animals must be purely the result of an evolutionary development. These higher types have not only developed their vascular organization and function, but survive because they can prey for a part of their life's energy on lower non-functioning forms, and have glands which liberate *archusia* into their circulating medium.

By these observations it has been possible to throw light not only on the nature of these formative mechanism of development and the evolution of function in higher animals, but also on the biological significance of vitamins, and to give further proof that cancer is nothing more than the result of an abnormal arrangement of cells. Normal development proceeds when a normal growth stimulus is transmitted through the blood stream where it acts to cause a primary development of blood vessels and secondary development of the tissues. Cancer develops when the same stimulus is placed outside the blood vessels or into the tissues so that the cells grow to form a densely cellular and non-vascular tissue. We have shown that coal tar acts in the same capacity, not by stimulating cells to proliferate and thus to form the non-vascular and cellular tissue, but by attracting cells from a wide area of the tissue and collecting them in dense masses about drops of it. Growth intervenes as these cellular masses become sufficiently large and stagnant.

¹¹ Supplied by The Vitamin Food Co., Westfield, Mass.

¹² Burrows, M. T., *Am. J. Physiol.*, 1917-18, xlv, 556.



CURVE No. 1. The three upper lines show the growth of rats fed on a diet containing butter and respectively *B. campestris*, *B. tumefaciens* and vegex. The two lower lines show the growth of rats on a diet containing *B. tumefaciens* and *B. campestris* respectively, but no butter.

WESTERN NEW YORK BRANCH

*New York Agricultural Experiment Station, Geneva, New York,
December 13, 1924.*

116 (2639)

**Heat from reactions between antigens and antibodies: Special
reference to diphtheria toxin and antitoxin.**

By STANHOPE BAYNE-JONES.

*[From the Department of Bacteriology, School of Medicine and
Dentistry, University of Rochester, Rochester, N. Y.]*

Although Arrhenius¹ calculated from the effect of temperature upon the equilibrium constant that 5480 calories would be produced by the reaction between 1 gram molecule of tetanolysin with 1 gram molecule of antilysin, no one has actually measured the amount of heat liberated by this or any similar reaction between an antigen and its antibody. The substances are not available in a pure state, and hence immunological units must be used in place of exact quantitative terms. This is unsatisfactory. Nevertheless, as a basis for future work, the heat produced by two types of antigen antibody reaction was studied by me in the laboratory of the Department of Pathology of the Johns Hopkins Medical School during the period 1920-1923.

The instrument used was the differential microcalorimeter devised by A. V. Hill.² A delicate thermocouple, a White potentiometer, designed for thermoelectric measurements, and a suitable galvanometer were employed to obtain the calorimetric readings in terms of microvolts. This type of calorimeter is admirably suited to the study of the heat of a relatively slow reaction, as the differential arrangement of the vacuum thermos flasks balances the temperature effects due to changes in the environment. The heat effects due to dilution, and unknown constituents in the fluids containing the antigens and antibodies can also be cancelled by an appropriate mixture in the flask, serving as a control to the flask containing the reacting fluids. In Hill's paper, to which reference has been made, the laws governing the cool-

¹ Arrhenius, S., *Immunochemistry*, 1907, p. 181.

² Hill, A. V., *J. Physiol.*, Cambridge, 1911-12, xliii, 261-285.

ing of these flasks, the method of finding their coefficients of temperature loss, by which they can be adjusted to lose or gain temperature at the same rate, the method of correcting for loss of temperature and of computing the total heat evolved are set forth in adequate detail, and will not be repeated here.

After calibration of the apparatus, testing the heat production of known chemical mixtures and after a verification of the necessary controls, the heat produced by the union between diphtheria toxin and antitoxin, and by the agglutination of bacteria by an immune serum were measured.

Two preparations of diphtheria toxin and two preparations of antitoxic serum and pseudoglobulin were mixed in differing proportions, and the production of heat by their reaction was observed. The reaction was found to be exothermic. The average value obtained was based upon the heat liberated by the complete saturation of 1 unit of antitoxin by sufficient toxin to equal, in antigenic content, the equivalent by flocculation of 1 unit of antitoxin. In terms of the unit recently introduced by Glenny and Okell,³ 1 unit of antitoxin combining with 1 L_t amount of toxin liberated 0.0645 gram calories.

At present, it is not possible to reduce this value to a more exact quantitative basis, as the amount of active substance, in grams, in 1 unit of antitoxin is not known, and the weight of 1 L_t amount of toxin is equally unknown. From the available data, however, it seems that the amount of heat produced, as found by these experiments, is a large amount. It is probably more of the order of that produced by many chemical reactions than of the magnitude of the heat produced by the mutual flocculation of oppositely charged colloids, as measured by Kruyt and van der Speck.⁴

Heat is evolved also when dead bacteria are agglutinated by an immune serum. Observations upon this effect were made upon a suspension of *B. typhosus* mixed with the serum of an immunized horse. Heat production during this reaction took place in two periods; the first corresponded to the period of the union of the bacterial antigen with the antibody, the second to the

³ Glenny, A. T., and Okell, C. C., *J. Pathol. and Bacteriol.*, 1924, xxvii, 187-200.

⁴ Kruyt, H. R., and J. van der Speck, *Kolloid-Zeitschrift*, 1919, xxiv, 145-155.

period of mechanical flocculation of the bacteria. The heat liberated by the combination between agglutinin and the antigen in this bacterial suspension, which consisted of 19×10^9 bacteria in saline, was 29 gram calories. The heat liberated by the clumping and flocculation of these bacteria was 10.8 gram calories. The immunological units employed in this instance have no quantitative significance.

Summary

The differential microcalorimeter was found to be suitable for the study of the heat produced by reactions between antigens and antibodies. Approximate values, which cannot be given definite quantitative meaning because of the nature of the crude solutions which must be employed, were obtained for the first time as follows:

The heat produced by the combination between 1 unit of diphtheria antitoxin and its equivalent L_t amount of diphtheria toxin was found to be 0.0645 gram-calories.

The reaction between dead typhoid bacilli and their specific agglutinin was also exothermic. Heat is produced during this reaction in two periods; the first corresponding to the phase of the union of the antigen with the antibody, the second to the clumping and flocculation of the bacteria.

117 (2640)

The weight curves of castrated kids.

By PIERRE A. FISH.

[*From the Department of Veterinary Physiology, Cornell University, Ithaca, N. Y.*]

Twin kids, born February 5, 1923, were used for the experiments. One, without horns, was used as the control; the other with horns was castrated at the age of 74 days. Later another kid with horns born April 1, 1923, was castrated at the age of 83 days and was added to the experiment. The age at which the operation was performed, it is believed, caught the young goats at about half way to the age of puberty. Daily records (except Sundays) were kept of the weights since the operations.

Weekly averages were obtained, and another average based upon four of the weekly averages was taken as the average for an approximate month.

For a period of eight months after the operation in the case of the first castrate, and of nine months after the operation in the case of the second castrate, there was apparently just as favorable growth in the castrates as in the control. From this point onward for nearly a year, there has been no material gain in the weight of the castrates although there have been minor fluctuations. The growth of the control, as evidenced by his increased weight, continued for seven months longer, when he weighed about 100 percent more than the castrates. Reckoning from the time of the operation, the growing period for the control lasted fifteen months while that for the castrates lasted only eight and nine months respectively. The removal of the testicles had abbreviated the growing period of the castrates by about seven months as compared with the control. Fig. 1.

During the eighth month it was discovered that the first castrate and the control had been deprived of salt for probably two or three weeks. (The salt brick had broken and the pieces were covered by the bedding.) During this period the weight of both goats diminished but gradually returned to the original level after the salt was renewed. The second castrate received salt continuously, and there was no depression of his weight at this time. During the ninth month, the second castrate was afflicted

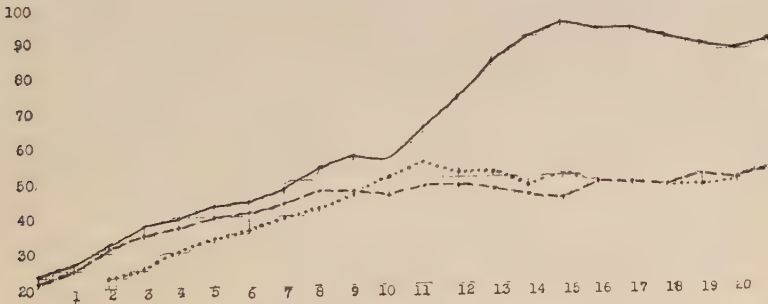


FIG. 1.

The figures in the vertical column represent the weight in pounds. The figures on the horizontal line represent the time in approximate months (four-week units). The curves begin just after the operation was performed. The continuous line is the curve of the control; the interrupted line that of the first castrate, and the dotted line that of the second castrate. In the case of the first castrate the curve shows that an approximate level has been maintained since the eighth month and in the case of the second castrate since the ninth month.

with a dermatosis (probably mange) and from this point his weight diminished over quite an extended period. Both of these events—salt deprivation and the dermatosis—occurred at the end of the growing period of the castrates. Whether these events had an influence in checking their growth is not known. The effect of salt deprivation upon the control was only temporary, for his weight increased rapidly for some months afterward. The first castrate was also afflicted with the dermatosis shortly before the second castrate, but the control was exempt from this trouble although all three were equally exposed. Since the dermatosis appeared only in the castrates it would appear that their power of resistance had been materially weakened by castration.

Reckoning from the date of the operation the control reached his maximum gain of 300 percent increase in about fourteen months. The first castrate at the eighth month had gained 120 percent; after various fluctuations he has reached 122 percent and 125 percent, and recently, probably as a result of endocrine treatment, he has attained a maximum of 143.4 percent. The second castrate reached his maximum gain of 140 percent at the ninth month and has not since returned to that level.*

The influence of the endocrine secretions upon growth is quite generally recognized and has been pretty well established in the case of the pituitary and thyroid glands. The results of this experiment would indicate that the internal secretion of the testes is also concerned as a factor in growth, although the effects may vary according to the age at which castration is performed. The fact that growth continued satisfactorily and at an equal rate with that of the control for several months after the operation may indicate that the loss of the interstitial secretion is compensated for by an increased activity of other endocrine organs, that finally this compensation fails and when this occurs subsequent growth is retarded.

One experiment although long extended, is not conclusive, but the fact that both castrates reacted to the operation in a similar way is significant.

*In his paper on "The Effect of Thyroidectomy on Growth in the Sheep and Goat as Indicated by Body Weight" (*Quarterly Journal of Experimental Physiology*, Vol. xiv, pp. 161-183, April, 1924) Sutherland Simpson shows growth curves, some of which are very similar to those obtained from the castrated kids.

118 (2641)

The utilization of fat in diabetes.*

By W. R. BLOOR and ETHYLN M. GILLETTE.

[From the School of Medicine and Dentistry, University of
Rochester, Rochester, N. Y.]

It is a well attested fact that in severe diabetes there is a pronounced tendency for the fat of the blood to be abnormally high (using the term fat to include all compounds of the fatty acids). In certain instances the fatty compounds may increase beyond the ability of the blood to carry them in solution and there is produced a visible milkiess, while in extreme cases the fat may reach very high figures—twenty percent and over having been reported. In a discussion of diabetic lipemia some time ago¹ evidence was collected which indicated that the lipemia of diabetes was the result of a diminished outflow of fat from the blood, directly or indirectly connected with the pancreatic insufficiency. Direct proof of diminished ability of the diabetic organism to utilize fat was however lacking, and it was with desire to obtain positive evidence on this point that the following work was undertaken. Experiments to elucidate this point were planned and carried out by Allen² and Wishart some years ago, but owing to loss of the data during the war they were never recorded. The subject is one of great practical importance, for while insulin has provided a means of abolishing the acute symptoms and saving life it does not cure diabetes, and its continued administration especially in large doses is, to say the least, inconvenient. Recourse must still be had to diet, and since fat is one article of food that does not form carbohydrate—or does so least readily—the use of high fat diets in diabetes has had a considerable vogue (Newburgh and Marsh, Petren) notwithstanding the warning of Allen that while good results may be obtained temporarily there is great danger of disaster.

The experiments were planned so as to provide a direct comparison between the effects of fat on the blood lipoids in a normal

* This work was made possible by a gift from Mr. and Mrs. W. H. Robinson of Pittsburgh.

¹ Bloor, W. R., *J. Biol. Chem.*, 1921, xlv, 201.

² Allen, F. M., *J. Metab. Res.*, 1922, ii, 219.

animal and in the same animal rendered diabetic by removal of most of the pancreas. Normal young adult dogs were taken and their blood reaction to fat studied by a series of feedings with analysis of the blood lipoids by the micro methods. About nine-tenths of the pancreas was then removed† leaving the part around and near the main duct, and, after recovery, the fat feedings were repeated. The diet after the operation was high protein and low fat. Sugar appeared in the urine at once after the operation. The two animals were killed to obtain the blood for gross study, one after seven weeks when moribund, and the other after two months while still in good condition although thin. The effect on the utilization of fat appeared only after a month, and showed itself not on the digestion and absorption, which apparently proceeded normally, but in the greatly increased reaction to the fat feeding as exhibited by the much greater and more persistent increase in the free fat of the blood plasma. The other lipoid constituents did not appear to be much affected nor were the corpuscle lipoids. In one animal (24-6) there was persistent lipemia (total fatty acid 3 percent) during the last week of life.

A table illustrating the significant effects is given below:

† I am indebted to Dr. John R. Murlin, director of the Department of Vital Economics, of this University, for these operations.

Fat Feeding Experiments.

Dog 24-6 before operation		Plasma mgm. per 100 cc.			
	Time	Cholesterol	F'ty Acids	Lecithin	Free Fat
Date, 9/11/23	8:45	52	470	406	183
Weight 29 lbs.	10:50	106	505	486	146
70cc. olive oil at 9:15	12:35	141	545	518	152
after one day fasting	2:35	150	692	544	280
	4:20	135	560	504	179
After operation					
Date, 6/24/24	8:45	88	510	296	283
Weight 22 lbs. 8 oz.	10:40	120	683	410	369
100cc. olive oil, 9:15,	12:40	107	1580	506	1260
after one day fasting	2:35	174	1440	478	1074
Total sugar output for day 46.1 gm.	4:30	199	1505	468	1127
Next day before feeding		148	635	408	314
Dog 24-7 before operation		Plasma mgm. per 100 cc.			
	Time	Cholesterol	F'ty Acids	Lecithin	Free Fat
Date, 9/27/23	9:45	129	620	466	267
Weight 26 lbs. 5 oz.	10:35	150	652	248	436
225 cc. olive oil af-	12:35	180	645	256	415
ter one day fasting	2:35	156	654	232	446
	4:20	188	640	224	427
After operation					
Date, 6/26/24	8:40	175	565	588	115
Weight 24 lbs. 6 oz.	10:40	171	1190	430	847
100 cc. olive oil af-	12:40	288	1530	468	1122
ter one day fasting	2:30	260	1430	448	1045
Total output sugar 54.9 gm.	4:25	159	985	480	612
Next day before feeding		156	610	346	328

The results show that in these animals, removal of enough of the pancreas to render them diabetic resulted in a much greater accumulation in, and slower removal of fat from the plasma than when a normal amount of pancreas was present. Further experiments are of course necessary to show whether the effect is general in diabetic animals, but the experiments indicate that these animals may not be able to utilize fat as well as normals.

119 (2642)

On the carbohydrate metabolism of malignant tumors.

By CARL F. CORI and GERTY T. CORI.

*[From the State Institute for the Study of Malignant Disease,
Buffalo, N. Y.]*

The free sugar, lactic acid and glycogen content of spontaneous and transplanted mouse carcinoma and of Jensen rat sarcoma has been investigated under various conditions. It was found that the free sugar content of these tumors was lower than that of any other tissue of the mouse or the rat so far investigated. Thus 16 tumors showed as an average 0.047 per cent free sugar, the maximum being 0.069 and the minimum 0.036. Comparative values for mouse organs have been obtained on a former occasion and showed as an average: liver, 0.300 per cent; muscle, 0.079 per cent; kidney, 0.126 per cent; brain, 0.059 per cent. Glucose administration had a marked effect on the free sugar concentration of the tumors. The tumors were analyzed 15, 30 and 60 minutes after giving glucose intraperitoneally. After 15 minutes the free sugar values were from 0.287 to 0.176 per cent at a blood sugar level from 0.634 to 0.396 per cent. After 30 minutes the free sugar values were from 0.311 to 0.184 per cent at a bloodsugar level from 0.464 to 0.230 per cent. After 60 minutes the free sugar values were from 0.078 to 0.057 per cent at a bloodsugar level from 0.194 to 0.166 per cent. A hyperglycemia produced by epinephrin also raised the free sugar concentration of the tumors.

The lactic acid values of 8 individual tumors ranged from 0.013 to 0.089 per cent, average 0.038 per cent. Comparative lactic acid values for other mouse tissues were: liver, 0.051 per cent for mice starved from 0 to 2 hours, and 0.011 for mice starved from 17 to 22 hours; muscle, 0.112 per cent. The lactic acid content of the tumors was considerably increased after glucose administration. Thus in 4 cases, where the tumors constituted from 2.7 to 22 per cent of the body weight of the animals, values of 0.117, 0.166, 0.160 and 0.141 per cent lactic acid were obtained; average 0.146.

The glycogen in 6 tumors varied from 0.122 to 0.303 per cent, average 0.196 per cent.

Warburg and co-workers^{1, 2, 3, 4} have shown that tumor tissue *in vitro*, in the absence as well as in the presence of oxygen, had an unusually large glycolytic power. They also found that the glycolysis was bound to the structure and did not take place in the liquid medium of the cells, which made it very probable that the energy derived from the splitting of glucose into lactic acid was utilized by the tumor cells, in other words, that the phenomena observed *in vitro* were also taking place *in vivo*. Our experiments support the idea that tumor tissue splits very large amounts of glucose into lactic acid under *vivo* conditions. Warburg found that the rate of glycolysis of tumor tissue *in vitro* was increased with increasing glucose concentration. Thus raising the sugar concentration from 0.04 to 0.2 per cent nearly doubled the rate of glycolysis, while just above 0.2 per cent glucose the rate was already at its maximum. A similar increase in the rate of glycolysis of the tumor tissue seems to occur in the living animal, when the sugar concentration of the tumors is raised from its resting level of 0.047 per cent to 0.2 per cent and more by the administration of glucose, since the lactic acid concentration in the tumors is raised 3 to 4 times after the glucose administration.

¹ Warburg, O., and Minami, S., *Klin. Wochenschr.*, 1923, ii, 776.

² Warburg, O., *Bioch. Z.*, 1923, cxlii, 317.

³ Minami, S., *Bioch. Z.*, 1923, cxlii, 334.

⁴ Warburg, O., Posener, K., and Negelin, E., *Bioch. Z.*, 1924, cvii, 309.

CALIFORNIA BRANCH

Stanford University Medical School, San Francisco, Cal.,

December 17, 1924

120 (2643)

A note on the refractive index of chitin.

By L. B. BECKING and JOSEPH C. CHAMBERLIN.

[*From the Laboratory for Economic Biology, Stanford University, Cal.*]

Chitin was prepared according to Krawkow's method¹ from crab, grasshopper and cicada carapaces. It was next dissolved in a large amount of twice normal HCl. This solution was gradually diluted with water until a flocculent precipitate appeared. This was filtered off, washed and dialized in goldbeater's skin. The dried product had a somewhat resilient, gutta-percha-like consistency.

The refractive index of this chitin was determined by Schroeder van der Kolk's immersion method as used in mineralogy.² The value proved to be the same for crab and insect chitin; $n_d = 1.525 \pm .005$.

This explains why delicate chitinous objects invariably almost completely disappear when mounted in Canada Balsam. Since this latter medium has a refractive index of 1.528-1.537 for sodium light, it is evident that good results can never be secured from preparations of unpigmented, unstained chitinous objects. Proteins are cleared ultimately in balsam, hence protein-impregnated chitin will almost completely disappear in balsam mounts.

Structural details in colorless insects can be very well observed in such liquids as Styrae balsam ($n_d = 1.630$). This resin is a logical mounting medium for chitin.

Permanent insect stains have to be chitin stains. It was found that a silver impregnation could be effected by light reduction of .1M silver nitrate solution. The chitin has to be pre-soaked in weak alkali, however, before the silver will "take". No definite iso-electric point could be determined either by silver nitrate or acid fuchsin method.³

¹ *Ztschr. f. Biol.*, 1892, xxix, 177.

² Ambrohn, H., *Anleitung z. Benutzung des Polarisations-mikroskop*, 1906.

³ Loeb, J., *Proteins and Colloidal Behaviour*, 1923.

121 (2644)

Quantitative changes in arterial blood sugar during canine anaphylactic shock.

By MARGERY McCULLOUGH. (Introduced by W. H. Manwaring and R. E. Swain).

[*From the Laboratory of Bacteriology and Experimental Pathology, and the Laboratory of Biochemistry, Stanford University, California.*]

Following the observation that glycogen disappears almost quantitatively from the canine liver during the first fifteen minutes of typical anaphylactic shock,¹ we have determined the parallel quantitative changes in blood sugar. The determinations were made by the Meyers-Bailey modification of the Lewis-Benedict technique.

Blood from non-etherized normal dogs shows a sugar content varying from 0.10 percent to 0.12 percent. On etherization the blood sugar rises to 0.13 percent to 0.18 percent, an average of 0.15 percent in our series. The sugar remains fairly constant at this level, with maximum variations of 0.03 percent.

Two control non-sensitized dogs (morphine-ether anesthesia), and two dogs giving only slight suggestions of horse serum sensitization (by Kymograph readings) were injected intravenously with 1 cc. to 2 cc. horse serum per Kg. of body weight. The blood sugar remained almost constant in these animals for thirty minutes, with maximum variations of 0.02 percent above or below the initial readings.

Four dogs giving typical anaphylactic shock were injected with 1 cc. to 2 cc. of horse serum per kg. of body weight. In all four the blood sugar increased rapidly in amount, reaching a maximum of 0.25 percent to 0.27 percent in from ten to thirty minutes, an average increase of 0.10 percent above the initial readings.

These percentages are practically identical with the thirty minute blood sugar readings in normal dogs, after intravenous injection of glucose equivalent to the total estimated glycogen content of the liver.

¹ O'Neill, F. L., Manwaring, W. H., and Moy, H. B., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 124.

122 (2645)

The Schultz-Dale technique with large guinea pigs and with calcium-free Fleisch-Ringer solution.

By P. BRISTOL and E. C. FLEISCHNER.

[From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco, Cal.]

In the course of various studies on sensitization it was found desirable to use large guinea pigs. It was noted that a calcium-free Fleisch-Ringer¹ bath at 37° C. reduced the amplitude of the contractions of excised uteri of guinea pigs weighing as much as 525 grams, and rendered these tissues suitable for anaphylactic studies. The application of this solution to the Schultze-Dale technique was suggested by the work of Ransum² dealing with the effect of strophanthus on cats' uteri suspended in a calcium-free physiological salt solution.

The modified Fleisch-Ringer solution has been in use for the last six months and has given uniformly satisfactory results with tissues of guinea pigs weighing not more than 600 grams. The effect of potassium-free as well as calcium-free solutions have been compared with that of the complete solution. Some of the results are shown in Charts 1-5.

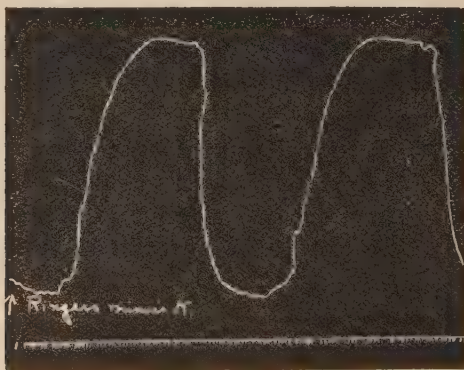


CHART I. Excised uterine horn from a guinea pig weighing 351 grams in a bath of potassium-free Fleisch-Ringer solution at 37° C. Ten second time interval recorded in all charts. Showing normal uterine contractions making observations impossible.

¹ *Arch. f. exper. Path. und Pharm.*, 1922, **xciv**, 22.

² *J. Pharm. and Exper. Therap.*, 1920, **xv**, 181.

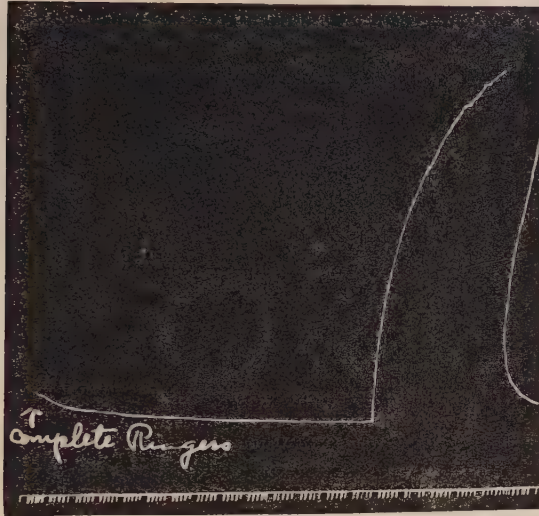


CHART II. Uterine horn from guinea-pig weighing 300 grams suspended in complete Fleisch-Ringer solution at 37° C. Also showing normal contraction in uterus of large guinea pig.

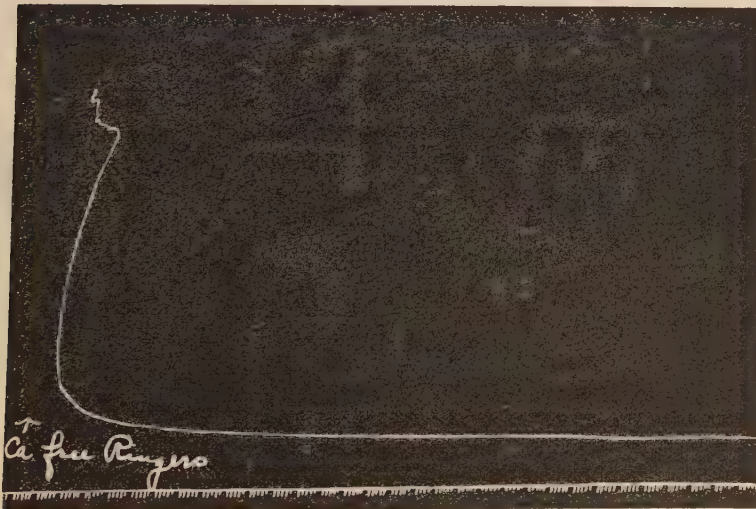


CHART III. Same horn as in Chart II with complete solution replaced by a calcium-free Fleisch-Ringer solution at 37° C. Contractions of uterus ceased, permitting use of Schultz-Dale technique.

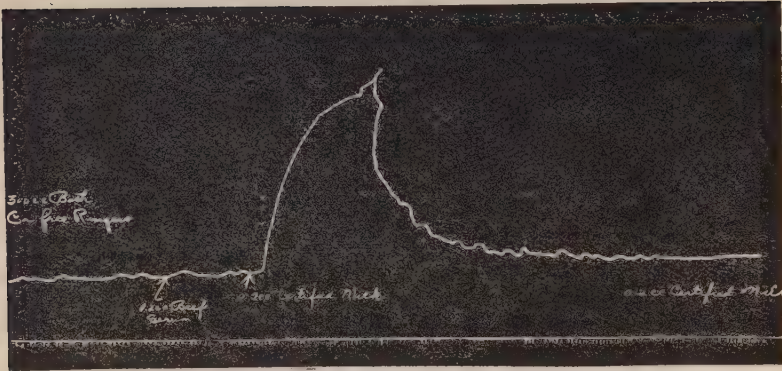


CHART IV. Weight of guinea pig 525 grams. Sensitized by feeding certified milk. Calcium-free Ringer's solution. Typical reaction on addition of certified milk.



CHART V. Guinea pig fed Horlick's malted milk and injected with 0.5 cc. toxin-antitoxin mixture subcutaneously to prevent rapid gain in weight and thus sensitized to horse serum. Weight 409 grams. Calcium-free solution. Typical reaction on addition of horse serum.

PEKING BRANCH

Peking Union Medical College, Peking, China, December, 1924.

123 (2646)

**A morphological distinction between infective larvae of
Ancylostoma and *Necator*.**

By RUTH M. SVENSSON.

[*From the Parasitology Laboratory of the Peking Union Medical College, Peking, China.*]

Infective larvæ of *Necator americanus*, *Ancylostoma duodenale* and *Ancylostoma caninum*, in which the stored food granules were reduced till the internal organs were clearly visible, were studied for morphological distinctions. The reduction of the food granules was obtained either by keeping the larvæ in soil cultures for 5 weeks and more, or by aging them artificially through increased activity. The latter method was devised by F. K. Payne (1923) and consists in burying the larvæ at the bottom of a jar filled with moist sand. The larva will migrate in great numbers up to the surface and during this time of activity rapidly use up their stored granules.

In these attenuated larvæ a difference at the point of union between the oesophagus and intestine was found to exist between larvæ of *N. americanus* on the one hand and those of *A. duodenale* and *A. caninum* on the other hand, indicating the probability of this being a generic distinction.

When *Necator* larvæ are studied under a 16 mm. objective, it appears as if the oesophagus and intestine were separated by an open space distally marked off by a definite transverse line, while in *Ancylostoma* larvæ the intestine is seen to follow as a direct continuation of the oesophagus. Careful study with an oil immersion objective reveals the fact that the lumen of the oesophagus is connected with the lumen of the intestine through a fine canal surrounded by a thickened part of the wall. This apparently functions as a sphincter. In *Ancylostoma* larvæ the inner outline of this sphincter follows as a continuous curve into the inner outline of the intestinal wall. *Necator* larvæ have the distal

part of the sphincter protruding into the lumen of the intestine in the form of a papilla separated from the intestinal wall by a groove. In high and in low focus the proximal arch of this groove appears as a transverse line at the base of the papilla. It is this line which stands out so strikingly under low magnification when the whole thickness of the larva is near focus at the same time and the optical impression of the part of the arch behind as well as the part in front of the papilla reaches the eye of the observer simultaneously. The sphincter itself being of a very low refractive index gives the impression of an open space between the transverse line mentioned above and the highly refractile distal part of the oesophagus.

124 (2647)

The specific effect of pneumococcus soluble substance on the growth of pneumococci in normal serum-leucocyte mixtures.

By RICHARD H. P. SIA. (Introduced by O. H. Robertson).

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City, and the Department of Medicine, Peking Union Medical College, Peking, China.*]

In a previous communication¹ a method was described for demonstrating the growth-inhibitory and bactericidal action of normal serum-leucocyte mixtures for the pneumococcus. Serum and washed leucocytes, contained in small tubes, were seeded with varying numbers of pneumococci. The tubes were then sealed with paraffine and attached to a specially devised apparatus which produced constant agitation during incubation. By means of this method, it was found that the growth of small numbers of pneumococci of low virulence, for cats, was markedly inhibited by the cat serum and leucocytes. On the other hand, small numbers of a strain virulent for rabbits, were able to grow in the rabbit serum-leucocyte mixtures.

By employing avirulent strains of pneumococci for rabbits and cats with the same method, a study has been made to determine

¹ Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **xxxix**, 219.

the action of the pneumococcus soluble substance^{2, 3, 4} on the growth of such strains of pneumococci in the rabbit or cat serum-leucocyte mixtures. It was found that whereas the growth of a small number of avirulent pneumococci was normally inhibited by such mixtures, the addition of a very small amount of the purified soluble substance of the homologous type was able to cause growth of the organisms in them. Such action of the soluble substance was shown to be highly specific to type. A type II substance assisted the growth of only type II pneumococcus, likewise a type III substance, the growth of type III pneumococcus only.

Experiments employing broth filtrates of young pneumococcus cultures in place of the purified soluble substances gave similar results, thereby established the identity of the purified substance with the substance originally described in the cultural fluids of pneumococcus cultures.

From the results of previous studies, such action of the pneumococcus soluble substance may be interpreted as having the power of rendering an avirulent pneumococcus of the homologous type virulent.

125 (2648)

The effect of chemical preservation of eggs upon the stability of their vitamin contents.

By ERNEST TSO. (Introduced by O. H. Robertson).

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

The Chinese preserved eggs or "pidan" are produced on a commercial scale from fresh ducks' eggs, and are perhaps as much relished by the Chinese people as cheese is in Western countries. In preserving, each egg is coated with a layer about 7 mm. thick of a mixture containing pure soda 5, burned straw ash 25, table salt 4, slacked lime 40, and boiling water 26. This again is

² Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **xxvi**, 477.

³ Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1923, **xxxviii**, 73.

⁴ Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **lx**, 801.

covered with rice husks to prevent sticking. The eggs are laid in earthenware jars, sealed with wet clay, for a month. Both the white and the yolk are then coagulated. The white has turned dark brown and the yolk greenish gray with concentric rings of different shades of gray. These eggs are marketed with their coverings on and are usually consumed within six months of production. The taste of these eggs can only be very imperfectly described as somewhat caustic and piquant and the odor largely ammoniacal.

In the preserved egg there was, according to Blunt and Wang,¹ a marked increase in the ash content and the alkalinity of the ash; and a partial decomposition of the proteins and the phospholipoids resulting in an excessive production of free ammonia and in a diminuation of the yolk-fat. It was believed that these characteristic changes were brought about by the combined action of bacteria and enzymes as well as by the alkali preservative.

It should be of interest, both from the point of view of setting a vitamin value to "pidan" as a food and the point of view of studying the properties of vitamins, to know what effects the preserving agents and the chemical changes have upon the stability of the presumably rich vitamin contents of the ducks' eggs.

Feeding experiments were made on 57 albino rats. Three or four animals were used for each single experiment. It was shown that 3 per cent of the preserved yolk, or 2 per cent of the ether extract of the dried preserved yolk contained sufficient amount of vitamin A to cure this vitamin deficiency disease in the rats. Five per cent of the preserved yolk was as efficacious as 5 per cent of liquid yolk from fresh ducks' eggs in curing Xerophthalmia, and inducing promptly the return of vigorous growth in rats which had declined in weight on basal diets deficient in vitamin A. On the other hand, 25 per cent of the preserved yolk exerted no appreciable influence on the course of vitamin B deficiency disease, though the yolk from fresh ducks' eggs was found to be very rich in vitamin B. Five per cent of the preserved yolk, or 2 per cent of its ether extract incorporated in the No. 84 Sherman and Pappenheimer diet, was effective in preventing and inducing healing of rachitic bone changes. Two per cent of the ether extract of the preserved yolk, after being oxidized for six hours by exposure to showers of heated air at a

¹ Blunt, K., and Wang, C. C., *J. Biol. Chem.*, 1916-1917, xxviii, 127.

temperature of about 120° C, was found to have lost its vitamin A content but retained to a large measure its anti-rachitic potency.

It seems justified to conclude that under the conditions as found in the Chinese preserved eggs, the originally rich vitamin B content is practically completely destroyed, but the stability of vitamin A and the anti-rachitic food factor is little or not at all affected.

126 (2649)

A method for the preparation of basal dietary free from vitamin A.

By ERNEST TSO. (Introduced by O. H. Robertson).

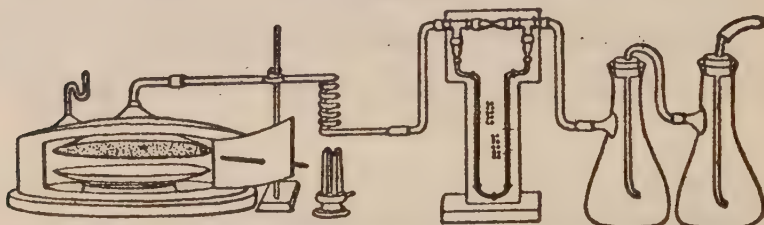
[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

The method depends upon the fact that vitamin A can be easily destroyed by oxidation and that the higher the temperature the more rapid, apparently, is the destructive process. The apparatus illustrated in the accompanying diagram consists of an iron coil of about 7 mm. bore; two suction flasks interposed between the coil and the compressed air faucet; one improvised Marshall and Kolls' flowmeter; a shower expansion of about 28 cm. diameter, made of copper sheet; and a galvanized iron drum box enclosing the shower expansion. Through a large window in the drum the food is placed under the air shower. Exit of the air current is provided for by a tube inserted in an upper corner of the drum.

By using a four tube burner under the coil and setting the air stream running at the rate of about three thousand liters per hour, the temperature of the food is maintained at about 110° C. in the case of yeast and, in the case of casein and starch, about 120° C. Two hundred grams of the food are spread over a 26 cm. aluminium tray placed at a distance of 6 cm. under the shower plate. The food is stirred at half an hour intervals in order to secure a uniform surface of exposure.

Experimental tests have demonstrated that beyond the desired effect the basal foods thus treated for 6 hours are not injured or unfavorably affected. The basal ration was composed of casein 20, starch 65, yeast 10, and McCollum's 185 salt mixture 5. In a separate test it was found that 5 per cent of yeast whether oxidized, untreated, or extracted with absolute alcohol was inadequate as the source of vitamin B. Fresh butter-fat of proved potency after a six hour exposure loses practically all of its vitamin A content as shown by its complete failure to prevent or cure vitamin A deficiency disease in the rats.

The advantages of this method over the usual absolute alcohol extraction procedure are, first, in the saving of a comparatively large item of expense otherwise involved in the use of absolute alcohol and, second, in the saving of much time and labor.



Apparatus for the preparation of basal dietary free from vitamin A.

SCIENTIFIC PROCEEDINGS.

NEW YORK MEETING.

Presbyterian Hospital, February 18, 1925.

127 (2650)

Pneumococci cultivation in large amounts.

By F. M. HUNTOON.

[*From the H. K. Mulford Biological Laboratories, Glenolden, Pa.*]

The need of large amounts of Pneumococci for certain immunological and chemical investigations led to the devising of the following methods:

The problem divided itself into three phases:

1. The discovery of a simple medium, easy to prepare and relatively inexpensive, which would allow of a vigorous growth of the organisms.
2. The devising of an apparatus permitting growth in bulk.
3. The recovery of the organisms from the culture medium.

THE MEDIUM.

The medium finally selected after much experimentation is known as the L. A. P. medium, the letters indicating the initials of the principal ingredients.

The formula is as follows: Tap water q. s.

Lactose -----	0.5	per cent.
Amnoids (Arlington Chemical Co.)----	0.2	per cent.
Peptone (Bacto) -----	0.1	per cent.
NaCl -----	0.25	per cent.
Dipotassium phosphate -----	0.5	per cent.
Monopotassium phosphate -----	0.03	per cent.

The ingredients are dissolved in the water with the aid of heat, and the solution sterilized.

The pH of the mixture should be 7.6 as tested cold with phenyl red.

In preparing large quantities of this medium it is necessary to sterilize the lactose separately, as a 10-15 per cent solution in distilled water. This avoids the caramelization of the lactose which acts as a growth retarder.

This medium must be inoculated heavily with a vigorously growing culture of the *Pneumococcus*. When such conditions are met the subsequent multiplication is very rapid.

Growth of one billion organisms to the cubic centimeter occurs in three hours, two billion in six hours, and from four to six billion in eighteen hours.

THE APPARATUS

The apparatus employed is so designed that it is used in the preparation of the medium, in its sterilization, and finally acts as an incubator during the growth of the *Pneumococci*.

It consists essentially of a conical cast iron tank with a capacity of 300 liters. This tank is jacketed and is lined throughout with acid proof enamel as is the tight fitting cover.

The jacket is connected with steam and water lines and a pipe system to ensure circulation of the water content when used as an incubator.

The cover is pierced with openings, for a thermometer, for a compressed air connection (used to stir up the contents of the tank), and an opening for the introduction of the inoculum.

In use, the L. A. P. medium is prepared in the tank in 200 liter amounts by adding all the ingredients with the exception of the lactose to the water. The temperature is raised to 110° C. by introducing steam into the jacket, and is held at this point for 2 hours. The steam is turned off and the tank allowed to remain hot overnight; on the following morning the temperature is reduced to 37° C. by passing water through the jacket. The lactose (previously sterilized in distilled water) is added, and the inoculum introduced.

The temperature is maintained at 37° C. by means of a single Bunsen flame acting on the pipe system spoken of before.

The pipe has its entrance to the jacket at one side of the upper level of the contained water, it is carried around to the opposite side and down to connect again at the lower level of the contained water, so that heat applied to this pipe causes a circulation through the pipe and the water jacket.

After 18 hours incubation the temperature of the culture medium is raised to 60° C. to kill any living *Pneumococci*, and

the growth of from four to six billion organisms per cubic centimeter is ready for recovery.

RECOVERY OF THE ORGANISMS.

This is accomplished by piping the growth to a battery of Sharpless super centrifuges, where it is centrifuged at 30,000 revolutions to the minute, which removes practically all the organisms from the medium.

The packed organisms are removed from the bowls of the machine and packed in glass containers, which are then placed at a temperature of minus five degrees C.

Organisms so obtained retain their morphology and staining characteristics, and are as useful for immuniological work as those obtained from the ordinary media employed.

This method has been in constant use for over a year and has proved satisfactory.

128 (2651)

The physiologic properties of some unsaturated hydrocarbons.

By LLOYD K. RIGGS. (Introduced by John F. Anderson).

[*From the Research Laboratories of E. R. Squibb & Sons, New Brunswick, New Jersey.*]

The recent introduction of ethylene and acetylene as anesthetics into medical practice has made it appear desirable that a general study of the physiologic properties of the unsaturated hydrocarbons be undertaken. Studies have therefore been carried out on hydrocarbons of the olefine, diolefine and acetylene series. Each hydrocarbon has been studied from the following points of view: 1. Symptoms produced in experimental animals by the inhalation of various concentrations of each hydrocarbon studied. 2. The toxicity of the various hydrocarbons when administered by inhalation. 3. An attempt has been made to relate quantitatively the anesthetic potency and the toxicity of the various hydrocarbons studied.

In order that these studies might be made as strictly comparable as possible a single strain of white rats of uniform weight was used.

The following concentrations of hydrocarbons induce anesthesia in fifteen minutes, and are hence considered to be of equal anesthetic potency: ethylene 90 per cent, propylene 40 per cent, butylene 20 per cent, amylene 6 per cent and acetylene 78 per cent. If the anesthetic potency of ethylene is taken as unity, the potencies of the others then become, propylene = 2.25, butylene = 4.50, amylene = 15.0 and acetylene = 1.15. On the basis of the potency of propylene as unity, the potencies of ethylene, propylene, butylene, amylene and acetylene then become 0.44, 1.0, 2.0, 6.6 and 0.52 respectively. Butylene and amylene cause a marked excitement stage, while ethylene, propylene and acetylene do not. Animals are prostrated by 20 per cent propadiene and by 5 per cent methyl acetylene in about 15 minutes, but the nervous symptoms produced by these hydrocarbons are so violent that they can scarcely be said to possess anesthetic properties.

The following concentrations of hydrocarbons cause respiratory failure in about two hours, and are hence considered to be of equal toxicity: propylene 65 per cent, butylene 20 per cent, amylene 6 per cent, acetylene 90 per cent, methyl acetylene 5 per cent and propadiene 15 per cent. On the basis of the toxicity of propylene as unity, the toxicities of these hydrocarbons are as follows: butylene = 3.25, amylene = 10.8, acetylene = 0.73, methyl acetylene = 13 and propadiene = 4.3. The toxicity of 95 per cent ethylene is probably more due to oxygen privation than to the toxic action of ethylene. Methyl acetylene is 18 times as toxic as acetylene.

The relationship between the potency and toxicity of each hydrocarbon may be expressed in the form of a ratio, thus:

$$\frac{\text{Potency} \times 100}{\text{Toxicity}} = \text{Index}$$

The index thus obtained may be called the index of safety or anesthetic index. Making use of the figures given above for the toxicity and potency of the various hydrocarbons on the basis of potency of propylene as unity, and of toxicity of propylene as unity, we obtain the following indices:

$$\begin{array}{l} \text{Propylene } \frac{1 \times 100}{1} = 100, \text{ butylene } \frac{2.0 \times 100}{3.25} = 61, \text{ amylene } \frac{6.6 \times 100}{10.8} = 61 \\ \text{and acetylene } \frac{0.52 \times 100}{.73} = 71. \end{array}$$

Hereditary visceral abnormalities in the descendants of
irradiated mice.

By HALSEY J. BAGG.

[From the Memorial Hospital and Cornell University Medical
College, New York City.]

About a year ago^{1, 2, 3, 4} the writer mentioned the occurrence of several mice with kidney defects that were descendants in the sixth and subsequent generations of certain x-rayed animals in which an attempt had been made to alter the germ plasm by physical agents. For details concerning the work leading to these studies, and the data so far recorded, the reader is referred to the references given below.

The present communication deals with the results of 1817 autopsies of the descendants of mice with visceral abnormalities, which are mainly of the urinary system but are also associated with other visceral disturbances. Preliminary genetic results are also given to show the nature of the inheritance of these abnormalities. The following is a brief summary of the types of visceral abnormalities so far observed with the number of individuals in each group recorded in parenthesis:

A. In adult animals:

1. Kidneys moderately unequal in size, with no hypertrophy of the larger (5).
2. One kidney nearly completely missing and hypertrophy of the other (3).
3. One kidney normal and the other pathological; polycystic kidneys (3), hydronephrotic kidneys (3).
4. Kidneys in reversed position, *situs inversus viscerum* (1).
5. Absence of one kidney (285). The solitary kidney is usually, but apparently not always, hypertrophied.

B. In 1142 animals examined within a few hours of birth:

1. Kidneys unequal in size (8).

¹ Bagg, H. J., and Little, C. C., *Am. J. Anat.*, 1924, xxxiii, 119.

² Bagg, H. J., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 228.

³ Bagg, H. J., *Am. J. Obstet. and Gynec.*, 1924, viii, 2.

⁴ Little, C. C., and Bagg, H. J., *J. Exp. Zool.*, 1924, xli, 45.

2. Congenital absence of one kidney (132). No hypertrophy of the solitary kidney was noted within 24 hours of birth.

3. Congenital absence of one kidney and reduction in size of the other (11).

4. Congenital hydronephrosis (9). In five instances the hydronephrotic kidney was solitary, while in 4 an apparently normal kidney was the mate of the abnormal one. A tortuous and much distended ureter is associated with this condition.

5. Congenital absence of both kidneys (149). The adrenal glands are apparently normal. The animals are born alive, of apparently normal size and activity, and live for about 24 hours.

Localized fetal hemorrhages of the viscera have been noted associated with kidney abnormalities in the following locations: region of the kidneys (3); liver (7); testis (9), there was one animal with a missing testis; pancreas (1).

C. Summary of anatomical results:

140 animals with right kidney missing; males 76, females 64.

145 animals with left kidney missing; males 77, females 68.

149 animals with both kidneys missing; males 85, females 64.

92 animals with one kidney missing and right eye abnormal.

101 animals with one kidney missing and left eye abnormal.

21 animals with one kidney missing and both eyes abnormal.

71 animals with one kidney missing and eyes normal.

40 animals with both kidneys missing and right eye abnormal.

59 animals with both kidneys missing and left eye abnormal.

10 animals with both kidneys missing and both eyes abnormal.

40 animals with both kidneys missing and eyes normal.

100 animals with the eye and kidney defects on the same side of the body.

96 animals with the eye and kidney defects on opposite sides of the body.

30 animals with the kidney, eye and foot defects present in the same individual.

D. Preliminary genetic results:

Crosses were made between normal albino female mice and males from the experimental strain showing kidney, eye and foot defects. The albinos were selected from a carefully inbred strain that I have had under my observation for about 12 years.

At the present time 41 first generation animals, all apparently normal, were obtained from mating 8 normal albino females with

3 males, each with a solitary kidney associated with eye defects as well.

Four hundred forty-one second generation animals were obtained from 52 *inter se* matings of first generation animals. The average number of young per litter was 8.4. One hundred and one, or approximately 20 per cent of these animals was abnormal, either in the kidney, eye or foot regions; and 340 were apparently normal in all respects. The expected 3 to 1 mendelian ratio in this instance is approximately 110.25 to 330.75.

The examination of the second generation animals was obtained from complete autopsies made at birth. One or both kidneys were missing in 60 of the 101 abnormal animals.

In back-cross matings between 16 first generation females and the abnormal male parent, 292 offspring were obtained from 50 matings. The average number of young per litter was 5.8. One hundred sixteen animals were abnormal in either the kidney, eye or foot regions, and 176 were apparently normal in all respects. Sixty-nine of the abnormal young had one or both kidneys missing. The records were made from autopsies at birth. The expected ratio of abnormal to normal animals in the above matings is 1 to 1. The results show, however, that 40 per cent of the back-cross animals were abnormal, and 60 per cent apparently normal.

130 (2653)

The metabolism of glycerol in phlorhizin diabetes.

By WILLIAM H. CHAMBERS* and H. J. DEUEL, JR.

[From the Physiological Laboratory, Cornell University Medical College, New York City.]

It has been commonly accepted that glycerol can be completely converted into glucose by the diabetic, although no convincing evidence for this has been published. The early experiments of Cremer¹ on a phlorhizinized dog and of Lüthje² on depancreatized

* National Research Fellow in Medicine (1924-25).

¹ Cremer, M., *München. med. Wchnschr.*, 1902, xlix, 944.

² Lüthje, H., *Deutsch. Arch. f. klin. Med.*, 1904, lxxx, 98.

dogs indicated that 40 per cent of glycerol fed was recovered as extra glucose in the urine. This figure has been used by McCann, Hannon and co-workers³ in the calculation of the antiketogenic value of glycerol in the diets of diabetic patients.

Five experiments have been conducted in this laboratory on three fasting phlorhizinized dogs to determine the amount of glucose formed from ingested glycerol. After giving to two animals (Dogs 51 and 57) 8.53 grams of glycerol (capable of yielding 8.33 grams of glucose), 8.07 grams and 8.20 grams of extra glucose were eliminated in the urine. This is a recovery of 96.9 and 98.4 per cent, respectively. We were unable to obtain as complete a recovery of extra glucose from the third animal (Dog 56), possibly on account of an incomplete absorption. The ingestion of 15.16 and 8.53 grams of glycerol resulted in a recovery of 55.9 and 53.8 per cent, respectively, while the subcutaneous injection of the smaller dose yielded 70 per cent.

A further demonstration that the glycerol is not oxidized in the phlorhizinized dog was obtained by a respiration calorimeter experiment on Dog 56, the animal in which an incomplete recovery of extra glucose had been noted. The respiratory quotient averaged 0.703 for the two hours preceding the ingestion of 8.53 grams of glycerol (which has a R. Q. of 0.857), and 0.678 for the three hours thereafter. Similarly, the heat production was unchanged by the ingestion of this substance.

These data give evidence that glycerol may be quantitatively converted into glucose in the diabetic animal.

³ McCann, W. S., Hannon, R. R., Perlzweig, W. A., and Tompkins, E. H., *Arch. Int. Med.*, 1923, xxxii, 226.

The influence of proteins on the diffusibility of calcium.

By ROBERT F. LOEB AND EMILY G. NICHOLS.

[From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.]

It was demonstrated last year¹ that when blood serum is dialysed against large amounts of *physiological salt solution*, the calcium of the serum is entirely diffusible at a pH of 7.4. When serum is dialyzed against *distilled water* brought to a pH of 7.4, part of the calcium is non-diffusible. When serum is dialysed against distilled water brought to a pH of 3 with HCl, calcium is again completely diffusible. It was suggested that the proteins, probably the globulins, were responsible for these variations in diffusibility.

In an attempt to prove this idea we have this year started to study the effect of pure solutions of euglobulin, pseudoglobulin and albumin on the diffusibility of calcium. The proteins were isolated from the ascitic fluid of a patient with cardiac insufficiency.

The work has not yet been completed because of experimental difficulties encountered in keeping solutions between pH 6 and pH 8 constant without large amounts of buffer. However, we have found that between these limits of pH, Ca is less diffusible the lower the hydrogen ion concentration in solutions of euglobulin and pseudoglobulin. Furthermore, we have found that the greater the concentration of these proteins the greater the amount of calcium which does not diffuse through the collodion membrane. To prove that the effect is not one of occlusion of the membrane by calcium or protein, a membrane was soaked one night in pseudoglobulin solution, and calcium chloride at a pH of about 10 was placed inside of the membrane and the solution was dialysed. The calcium, the initial concentration of which was 10 mg. per 100 cc., was completely diffusible.

Thus we see that euglobulin, pseudoglobulin and possibly serum albumin appear to alter the diffusibility of calcium through collodion membranes when dialyzed against water at a pH of 6 to 8. This is probably the result of the formation of Ca proteinate.

¹ Loeb, Robert F., *J. Genl. Physiol.*, 1924, vi, 453.

132 (2655)

The importance of changes in electrical charge in specific bacterial agglutination.

By GERALD S. SHIBLEY. (Introduced by W. W. Palmer).

[From the Department of Medicine of the College of Physicians and Surgeons of Columbia University, and the Presbyterian Hospital, New York City.]

We have undertaken studies of the changes in the electrical charge on bacteria that take place in specific bacterial agglutination. Last year,¹ working with pneumococci and paratyphoid bacilli, we were able to show that their immune agglutinating sera possess a specific charge-reducing effect which is quantitatively related to the agglutination titre of the serum, which may be removed by absorption of agglutinin by the homologous or-

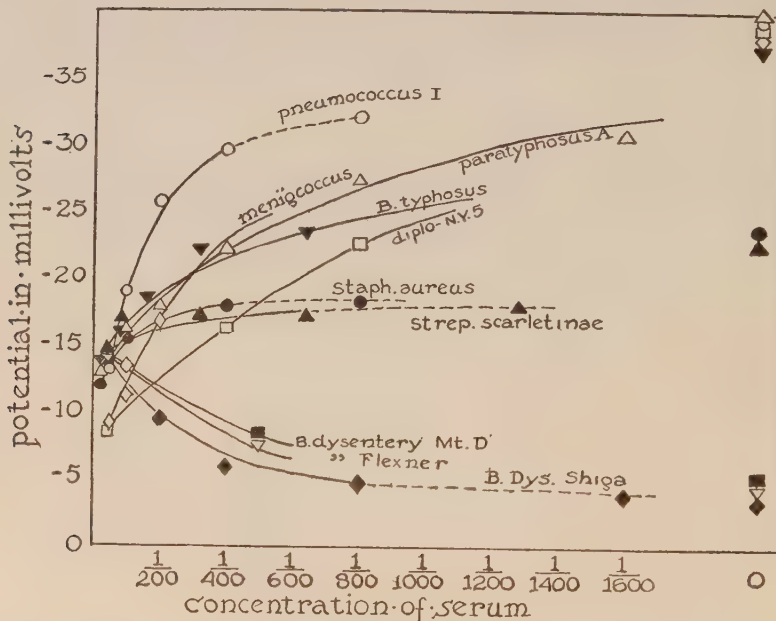


FIG. 1.

Effect of ten immune agglutinating sera upon potential and agglutination of their homologous organisms. Experiments in G. P. A. buffer pH 7.0 (M/200). Solid lines indicate agglutination, broken lines, no agglutination.

¹ Shibley, G. S., *J. Exp. Med.*, 1924, xl, 453.

ganism; this specific effect was not demonstrable in a highly protective, non-agglutinative serum.

This year, these studies have been extended to many other organisms and their sera; and it has been found that the conclusions set forth above may be enlarged by the statement that, where the natural charge on the organism is very low, specific serum *raises* the charge. Determinations were made (Fig. 1) of the changes in charge produced by their specific sera upon Type I pneumococci, meningococci, staphylococci, hemolytic streptococci, a diplococcus variant of streptococci, typhoid, and paratyphoid A bacilli, and three strains of the dysentery bacillus, Flexner, Shiga and Mt. Desert. In the case of the first named seven organisms, where, under the conditions of the experiment, the charges without serum are high or relatively high (23-40 millivolts), the homologous agglutinating sera produce a reduction of charge which is directly proportional to the concentration of

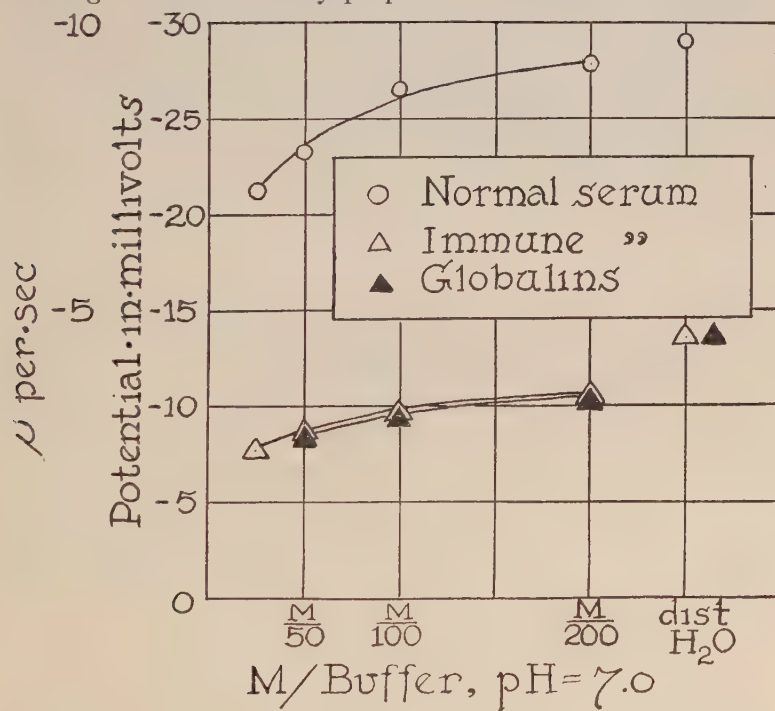


FIG. 2.

Effect of G. P. A. buffer pH 7.0 in varying molar concentration upon potential of Type I pneumococci treated with normal and immune serum 1:20 and of globulins of the immune serum.

the serum. However, with the three dysentery strains, where the natural charge is low (5 mv.) the effect of the specific sera is to raise the charge. In all cases, no matter what the initial charge, the effect of the serum in high concentration is to bring the charges of the bacteria to a *common potential level* (8-14 millivolts). It is known that bacterial agglutinins are included in the globulin fractions of immune sera. Euglobulin particles from human ascitic fluid were tested in electrolyte solutions similar to those used in the preceding experiments, and their charge falls into the potential zone noted above. Also, the globulin particles of a Type I pneumococcus serum were thrown down by the addition of distilled water, and the charges on these particles and on sensitized Type I pneumococci were compared (Fig. 2), and were found to be practically identical, *i. e.*, the sensitized bacteria act essentially like globulin particles, or in other words, the bacteria are coated by the agglutinin.

From these findings it may be concluded that the first step in the specific agglutination of bacteria is a selective coating of the organism by its particular agglutinin, that the changes in electrical charge accompanying the phenomenon are the result alone of this coating, and that the changes in charge, although perhaps contributory, do not, *per se*, have great importance in the mechanism of specific bacterial agglutination.

133 (2656)

Notes on the mechanism of paroxysmal hemoglobinuria.

By GEORGE M. MACKENZIE.

[*From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

The assumption that the Landsteiner¹ phenomenon constitutes the actual mechanism in paroxysmal hemoglobinuria has been questioned on clinical grounds, and has led to several attempts to demonstrate some other factor promoting intravascular hemoly-

¹ Donath, J., and Landsteiner, K., *München. Med. Wchnschr.*, 1904, li, 1590.

sis in this disease. Hijmans, van den Bergh and Hijmans,² and Hannema and Rytma³ have reported an activating effect on the hemolysin by CO₂. They found that, in the presence of CO₂, the hemolytic mechanism operates at room temperature. The effect was observed when the hemoglobinuric serum was set up with normal cells as well as with the patient's cells, and when the serum alone was exposed to CO₂ and afterwards mixed with the red cells and complement. The action of CO₂ therefore seemed to be on the hemolysin alone.

Two patients with this disease have recently been under observation. They are congenital syphilitics 5 and 8 years of age. One has frequent spontaneous attacks of hemoglobinuria even in temperatures above 16 degrees C. He has a low titer of hemolysin in his serum. The other has spontaneous attacks only after outdoor exposure on the coldest days of winter, or after immersion of an extremity in ice water. The hemolysin in his serum is present in a high titer. Hence in these patients the susceptibility to spontaneous attacks is inversely proportional to the titer of Landsteiner hemolysin in the serum, suggesting that some other factor in addition to the Landsteiner mechanism is operative. The presence of anti-complementary substances in the serum of the patient with a low hemolysin titer and high clinical susceptibility has been excluded. Repeated efforts to show with the blood of these patients that CO₂ acts *in vitro* as an activator of the Landsteiner hemolytic mechanism have been unsuccessful. The serum of each of these patients and mixtures of serum, cells and complement have been exposed at room temperature to concentrations of CO₂ varying from 3.5 per cent to 80 per cent with no hemolysis, upon subsequent warming to 37.5 degrees C. Nor has the titer of the hemolysin been any higher when the usual chilling test was carried out in the presence of CO₂ in concentrations from 20 to 40 per cent.

² Hijmans, van den Bergh, A. A., and Hijmans, C., *Berl. klin. Wchnschr.*, 1909, xlv, 1251.

³ Hannema, L. S., and Rytma, J. R., *Lancet*, 1922, (II), 1217.

134 (2657)

Ketogenesis following the feeding of ι -oxystearic ethyl ester.

By R. WEST and E. M. BENEDICT.

[*From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City.*]

This investigation was undertaken to attempt to determine whether or not an even carbon fatty acid with a negative group attached to the α , γ , ϵ or some corresponding carbon atom would yield acetone on oxidation in the body.

ι -oxystearic acid was prepared by the method of the Saytzeffs,¹ and esterified in the usual manner with absolute alcohol and dry HCl. The ester was then homogenized to a cream with skimmed milk. The acetyl value of the ester was 80-85. Urinary nitrogen was determined by Kjeldhaly's method, acetone by that of Van Slyke & Fitz, organic acids by that of Van Slyke and Palmer, creatine and creatinine by Folin's microchemical method. Stool fat was determined by Sharp's nephelometric method.

The appended tables show a definite reduction in urinary acetone when the synthetic fat was fed, and stool fat analyses indicate that it was absorbed by the body. It is of interest to note that though the acetone falls, organic acid output remains almost unchanged, and it seems probable that the synthetic product is at least in part oxidized and not wholly stored by the body. It will be noted that the synthetic fat was not quantitatively hydroxylated; but in one experiment not here tabulated (in which ι -oxystearic ethyl ester, having an acetyl value of but 40 was fed), there was no reduction in ketosis. It, therefore, appears that the non-hydroxylated fraction of the product fed is ketogenic.

¹ Saytzeff, M. C., and A.,

METABOLISM EXPERIMENTS.

I

S. ♂ 1924	DIET					URINE				
	Wt. lbs.	CHO	P	Fat Food l-ox.	Stool fat gm.	Fat absorbed gm.	Vol.	N- gm.	Ace- tone gm.	org. acids cc N/10 HCl
Sept. 27-28	151	54-60	185-	0	1350	8.88	623
28-29	151	54-60	185-	0	838	9.52	0.60	503
29-30	151	20-60	200-	0	1500	11.35	0.92	713
30- 1	152.5	20-60	200-	0	1380	10.45	6.44	1356
Oct. 1- 2	149.7	20-60	200-	0	2316	11.00	7.82	1572
2- 3	148.7	20-60	200-	0	12.7	187.3	1700	10.77	6.04	1382
3- 4	148	20-60	114-100		27.8	186.2	1590	10.90	3.56	1186
4- 5	148	20-60	200-	0	620	9.58	9.50	1106

II

W. ♂ 1924										
Sept. 27-28	125.5	54-60	185-	0	1350	12.60	786
28-29	125.0	54-60	185-	0	1920	12.35	2.64	938
29-30	124.5	20-60	200-	0	1710	11.62	2.52	980
30- 1	125.0	20-60	200-	0	1925	12.15	7.72	1538
Oct. 1- 2	123.0	20-60	200-	0	2140	10.25	9.30	1854
2- 3	122.5	20-60	200-	0	14.2	185.8	1295	12.10	7.50	1669
3- 4	122.0	20-60	119-100		35.6	178.4	1735	11.28	3.90	1220
4- 5	122.0	20-60	200-	0	1390	10.87	5.53	1152

III

P. ♂ 1924.										
Nov. 6- 7	182	77-70	204-	0
7- 8	181.5	77-70	204-	0	2325	12.7	535
8- 9	77-70	204-	0	2480	12.3	0.12	629
9-10	179	20-70	229-	0	1810	11.6	0.52	558
10-11	178	20-70	229-	0	2572	14.2	2.75	859
11-12	177	20-70	229-	0	2250	9.9	3.07	890
12-13	175.5	20-70	229-	0	6.7	222.3	3240	13.7	3.42	1038
13-14	176	20-70	105-150	} 22.8* 232.2*	2500	12.9	2.02	1088
14-15	175.5	20-70	105-150		2245	12.2	1.86	1099
15-16	175.5	20-70	105-150		2285	12.5	1.57	1086
16-17	175.5	20-70	229-	0	2525	11.4	2.46	816
17-18	175	20-70	229-	0	2360	11.2	2.40	853
18-19	174.5	20-70	229-	0	2860	11.2	2.68	907
19-20	174.5	20-70	170-	0	2260	11.3	1.28	761

* Per 24 hours, averaged from stools of 3 days, 14th through 16th.

We wish to express our thanks to Dr. H. D. Dakin, who suggested this problem, and whose advice has proven invaluable.

135 (2658)

Some reactions in sensitized guinea pigs to the filtrate of
scarlatinal streptococcus.

By A. R. DOCHEZ and LILLIAN SHERMAN.

[From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City.]

A large number of guinea pigs were given skin tests with the filtrate of a scarlatinal streptococcus, and no reaction was observed. It was found that the animals could be sensitized in various ways so that they would then give positive reactions to the filtrate.

One group of guinea pigs was sensitized by subcutaneous injections with the filtrate; a second group was sensitized with living culture injected subcutaneously; a third group was sensitized with killed cultures subcutaneously; and a fourth group was sensitized with killed culture intracutaneously injected.

The pigs receiving the living culture were not tested until the abscesses had healed; the remaining pigs were tested on the seventh to the eleventh days after the last injection. Skin tests were made repeatedly after that time to determine the period of time that the pigs remained sensitive.

Tests made a month after the last injection showed the reaction at its peak, although some of the pigs showed good positive reactions as early as the seventh day.

The pigs showing these positive reactions to the filtrate were also given an intracutaneous injection of neutralized toxin (equal parts of filtrate and antitoxin incubated for one hour), and the tests were negative. Here we have an acquired skin reaction with filtrate that is neutralizable.

The solubility product of tertiary calcium phosphate and its
importance in biological systems.

By L. EMMETT HOLT, JR., VICTOR K. LA MER, and H. BRUCE CHOWN.

[From the Department of Pediatrics, Johns Hopkins University,
Baltimore and the Department of Chemistry, Columbia University,
New York.]

Calcium metabolism in general is unquestionably connected with the solubility of $\text{Ca}_3(\text{PO}_4)_2$ and this in turn with the ions of H_3PO_4 and Ca^{++} through the solubility product principle. This is particularly true for the deposition and absorption of bone as well as for the many related pathological conditions. Inasmuch as $\text{Ca}_3(\text{PO}_4)_2$ cannot precipitate unless the ion product $[\text{Ca}^{++}]^3 \times [\text{PO}_4^{---}]^2$ exceeds the equilibrium value of this product (K_{sp}), it is necessary to evaluate K_{sp} under various conditions in order to determine the degree of saturation or undersaturation before proceeding to an investigation of the other factors involved in calcium metabolism. It appears from our studies that the K_{sp} of the tertiary phosphate of calcium is of more biological importance than the secondary phosphate CaHPO_4 . Data have been obtained on the latter and will be communicated later.

In a system containing orthophosphates, a knowledge of the hydrogen ion concentration and of the total phosphorus content makes it possible to calculate the concentration of (PO_4^{\equiv}) ion with reasonable accuracy, by using the formula:

$$[\text{PO}_4^{\equiv}] = \frac{[\text{P}]K_1K_2K_3}{[\text{H}^+]^3 + [\text{H}^+]^2K_1 + [\text{H}^+]K_1K_2 + K_1K_2K_3}$$

where $[\text{P}]$ represents the molar concentration of phosphorus as phosphate, $[\text{H}^+]$ that of hydrogen ion, and K_1 , K_2 , and K_3 the 1st, 2nd, and 3rd ionization constants of phosphoric acid, respectively. This formula is derived from the equations defining these three ionization constants:

$$(1) \frac{[\text{H}^+] \times [\text{H}_2\text{PO}_4^-]}{[\text{H}_3\text{PO}_4]} = K_1$$

$$(2) \frac{[\text{H}^+] \times [\text{HPO}_4^{--}]}{[\text{H}_2\text{PO}_4^-]} = K_2$$

$$(3) \frac{[\text{H}^+] \times [\text{PO}_4^{\equiv}]}{[\text{HPO}_4^{\equiv}]} = K_3$$

and the equation:

$$(4) [\text{P}] = [\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{\equiv}] + [\text{PO}_4^{\equiv}]$$

which expresses the fact that the molar concentration of total phosphorus [P] is equal to the sum of the molar concentrations of the unionized and ionized forms of phosphoric acid.

A knowledge of the PO_4^{\equiv} ion concentration and the Ca^{++} ion concentration enables one to calculate the value of the solubility product constant for tertiary calcium phosphate.

$$[\text{Ca}^{++}]^3 \times [\text{PO}_4^{\equiv}]^2 = K_{sp}$$

in systems which are in equilibrium with solid $\text{Ca}_3(\text{PO}_4)_2$. The system studied was prepared by titrating orthophosphoric acid with lime water, and determining the pH electrometrically after periods—extending in some cases to 8 months—had elapsed to insure equilibrium. Ca and P were determined for the solution; the composition of the solid phase was repeatedly checked by chemical analyses of the precipitates in equilibrium with the solution.

The true solubility or thermodynamic product for $\text{Ca}_3(\text{PO}_4)_2$ which is a constant under all conditions is the product of the activities or active masses of the Ca^{++} and PO_4^{\equiv} ions which may be written:

$$[a\text{Ca}^{++}]^3 \times [a\text{PO}_4^{\equiv}]^2 = aK_{sp}$$

At the present time, however, methods for measuring these individual ion activities with any degree of accuracy are not available; we have therefore confined ourselves to a study of the variation of the *stoichiometric* solubility product constant of the salt $\text{Ca}_3(\text{PO}_4)_2$:

$$[\text{Ca}^{++}]^3 \times [\text{PO}_4^{\equiv}]^2 = K_{sp}$$

from which one can evaluate the mean activities of the ions of the salt.

It was found that the stoichiometric constant is greatly affected by the salt content of the solution. Thus, in a system containing only calcium salts and sodium orthophosphates at high dilution, variations from 3×10^{-30} ($\text{p}K_{sp} = 29.5$) to 3×10^{-32} ($\text{p}K_{sp} = 31.5$) are obtained at 38°C . The chief variable responsible for these marked changes is apparently the concentration of primary

phosphate in the solution. The effect of foreign salts of different valence types on the solubility product constant is shown by the following table:

	K_{sp} at 38° C.	pK_{sp}	ΔpK_{sp}	$f \text{ Ca}_3(\text{PO}_4)_2$
No foreign salt. Extrapolated to infinite dilution.	1. $\times 10^{-32}$ (about)	32.+	—	1.00
M NaCl 8	2.44×10^{-30}	29.61	2.4	.33
M Na_2SO_4 8	4.73×10^{-29}	28.33	3.7	.18
M MgSO_4 10	5.97×10^{-26}	25.22	6.8	.04

The symbol pK_{sp} refers to the negative log of the stoichiometric K_{sp} of tertiary calcium phosphate as defined above, analogous to pH^+ . Larger values of pK_{sp} indicate that $\text{Ca}_3(\text{PO}_4)_2$ is more soluble. ΔpK_{sp}^+ is the difference between the true value; *i. e.*, the value of K_{sp} at infinite dilution where the gas laws hold rigidly, and the stoichiometric value at a given salt concentration. ΔpK_{sp} therefore is proportional to the activity of coefficient (f) of the salt by the general relation.

$$\Delta pK_{sp} = -\nu \log f \text{ (salt)} = -5 \log f \text{ (Ca}_3\text{(PO}_4\text{)}_2\text{)}$$

ν is the number of ions composing the salt.

Although the solubility product for $\text{Ca}_3(\text{PO}_4)_2$ is greatly affected by the addition of salts and proteins, it remains remarkably constant when a medium of constant salt composition is maintained. Thus in a solution containing inorganic salts in approximately the concentration found in blood serum, the solubility product constant at 38° C. was found to be very close to 6×10^{-28} ($pK_{sp} = 27.2$), and in blood serum itself a product of about 1×10^{-26} ($pK_{sp} = 26.0$) was regularly found at this temperature.

These marked salt effects are not surprising in the light of the studies of Bronsted and LaMer.¹ From a consideration of the high valences of the ions of the saturating salt, the magnitude of the effects may be predicted from the equation which they derived and tested. In the present case their equation 34 can be reduced to the simple form

¹ Bronsted and LaMer, *J. Am. Chem. Soc.*, 1924, xlii, 555.

$$\Delta pK_{sp} = 3\sqrt{\mu} + \beta\mu$$

where μ is the ionic strength of the solution as defined by G. N. Lewis² and β is an empirical constant depending primarily upon the size of the ions involved. The data show that the magnitude of the salt effect is in as good agreement as could be expected in view of the difficulties involved in studying the system and the unsymmetric nature of the valence types of the salts used. A critical investigation of the theory of Debye and Hückel upon which the equation is founded shows that a definite theoretical reason exists for the extension of the theory in this respect. Accurate data on appropriate salts are now being obtained for a test of a more general equation resulting from a consideration of the effects of unsymmetric valence types.

Since the stoichiometric solubility product remains constant in a medium like blood serum in which relatively small changes in the salt concentration (ionic strength) occur, it is obvious that this constant can be used to determine whether blood serum is supersaturated or undersaturated with $\text{Ca}_3(\text{PO}_4)_2$. Calculations which we have made of the ion product:

$$(\text{Ca}^{++})^3 \times (\text{PO}_4\equiv)^2$$

indicate that human serum is normally very much supersaturated with $\text{Ca}_3(\text{PO}_4)_2$. The ion products in normal serum and in cases of active and healing rickets are shown in the following table:

	$\text{Ca}^{++})^3 \times \text{PO}_4\equiv)^2$	P (ion product)
Active Rickets	3.5×10^{-26} to 8.4×10^{-25}	25.45 to 24.08
Normal (adults)	6.2×10^{-25} to $1. \times 10^{-24}$	24.20 to 24.00
Healing Rickets	6.5×10^{-25} to 4.8×10^{-24}	24.18 to 23.32

Even in active rickets the serum would seem to be considerably supersaturated, although not to the extent of normal serum.

When normal serum is shaken continuously at 38° C., no reduction in the ion product $[\text{Ca}^{++}]^3 \times [\text{PO}_4\equiv]^2$ occurs, even after 2 weeks. When, however, this shaking is carried out in the presence of solid $\text{Ca}_3(\text{PO}_4)_2$ a gradual deposition of this salt

² Lewis and Randall: "Thermodynamics and Free Energy of Chemical Substances," New York, 1923. Page 364.

occurs, and in about a week values close to that of the solubility product constant are obtained. This slow precipitation has been observed also in inorganic solutions. The rate of precipitation apparently depends upon how much the value of the solubility product is exceeded; *i. e.*, the rate of reaction is proportional to the free energy change ($-\Delta F$) or driving force of the process.

Thus one might expect to find that in active rickets there exists not complete arrest of calcification, but rather a marked diminution in the rate of calcification, resulting from the diminution of the ion product in blood serum. Some observations recently made on rats by Dr. Shipley² indicate that this is the case. If rats are kept a sufficiently long time upon a rickets-producing diet, a fine deposition of calcium in the metaphysis is often found. Moreover in human rickets a complete arrest of calcification is seldom if ever found.

The tendency of tertiary calcium phosphate to remain supersaturated in solutions for long periods of time would seem to be of considerable biological importance. It is by this mechanism that the blood is able to hold quantities of calcium sufficient to prevent tetany.

A more detailed report of these experiments will shortly be published.

137 (2660)

A new color test for differentiating neoarsphenamine from sulfarsphenamine.

By L. FREEDMAN and A. E. SHERNDAL. (Introduced by H. E. Dubin).

[*From the H. A. Metz Laboratories, Inc., Brooklyn, N. Y.*]

Although sulfarsphenamine, which was first introduced clinically in France as Sulfarsenol, is closely related to neoarsphenamine, a distinct chemical difference exists between the two drugs, the former being a derivative of sulfurous acid while the latter is a derivative of the hypothetical sulfoxylic acid.

Publications of Macallum,¹ deMyttenaere² and others indicate

² P. G. Shipley, personal communication.

that more or less uncertainty exists not only in the nomenclature of these drugs but also as to their chemical characteristics. It has also been found by various investigators that the trypanocidal and curative properties to which these drugs owe their practical importance, vary with these differences in chemical structure. It is therefore of great importance to be able to definitely distinguish between these two drugs.

Voegtlin and Johnson³ in 1922 suggested a color test for differentiating between neoarsphenamine and sulfarsphenamine. This test is based on Reinking, Dehnél and Labhardt's⁴ observations that compounds containing the group $-\text{CO}-\text{SO}\cdot\text{Na}$ reduced indigo carmine, while the compounds containing the group $-\text{CO}-\text{SO}_2\cdot\text{Na}$ did not reduce the dye. Voegtlin and Johnson³ found that an aqueous solution of neoarsphenamine will decolorize indigo carmine in a few minutes, if gently heated, yielding a yellow solution. Under the same conditions they found that sulfarsphenamine did not decolorize the dye.

A test, such as that described above, which requires heating is objectionable because of the ease with which compounds of the arseno type undergo decomposition at higher temperatures. We have also observed that some sulfarsphenamines, under the conditions described, will decolorize indigo carmine, especially when the solutions are acidified or made strongly alkaline.

We have found that by using methylene blue in place of indigo carmine, we have a reagent which gives a more specific test for differentiation of neo and sulfarsphenamine. This test, which has the added advantage of being workable at room temperatures, is carried out by adding a few drops of a $\frac{1}{4}$ per cent aqueous solution of medicinal methylene blue to a 1 per cent or stronger solution of the drug. With neoarsphenamine, the blue color of the dye is reduced in a few seconds to the colorless leuco base, whereas sulfarsphenamine fails to decolorize the dye.

Briefly summarizing our results, we have found that, under the conditions described above, methylene blue will be decolorized by:

¹ Macallum, A. Douglas, *J. Am. Chem. Soc.*, 1921, xliii, 643; *Ibid*, 1922, xliv, 2578.

² de Myttenaere, F., *Bull. acad. roy. med. Belg.*, 1923, (5) iii, 258.

³ Voegtlin, C., and Johnson, J. M., *J. Am. Chem. Soc.*, 1922, xlv, 2573.

⁴ Reinking, K., Dehnél, E., and Labhardt, H., *Ber. d. deutsch. chem. Gesellsch.*, 1905, xxxviii, 1069.

1. Neoarsphenamine.
2. Acid solutions of formaldehyde sulfoxylate (formaldehyde sulfoxylic acid).

3. Strongly alkaline solutions of salvarsan.

Methylene blue is not decolorized by:

1. Sulfarsphenamine.
2. Neutral or alkaline solutions of formaldehyde sulfoxylate.
3. Acid or slightly alkaline solutions of salvarsan.
4. Solutions of formaldehyde bisulfites, either acid, alkaline or neutral.

Work is now in progress on a titrametric application of this test which appears to give promise of indicating certain variations in commercial neoarsphenamines which compare favorably with certain chemical and biological characteristics of the different products.

138 (2661)

Group specific flocculation reactions with alcoholic extracts of human blood.

By K. LANDSTEINER, J. VAN DER SCHEER, and DAN H. WITT.

[From the Laboratories of The Rockefeller Institute for Medical Research and the Second Medical Cornell Division of Bellevue Hospital, New York City.]

In a previous communication it was stated that by adding inactivated hemolytic immune sera to emulsions of alcoholic extracts of blood, flocculation reactions can be obtained.¹

In continuation of our experiments, tests were made with rabbit anti-human blood immune sera. It was found that in this case also a certain number of the immune sera gave positive reactions under the conditions of our experiments. The immune sera were prepared by injections of Group I and of Group II blood corpuscles (American nomenclature).² Some of the most active anti-group II immune sera showed a distinctly stronger flocculation with the extracts of II corpuscles than with those of Groups I and III (as shown in the table). To make the emul-

¹ Landsteiner, K., and van der Scheer, J., *Proc. Soc. Exp. Biol. and Med.*, 1924, **xxii**, 170.

² *J. Am. Med. Assn.*, 1921, **lxxvi**, 130.

sions, 1 cc. of the filtered alcoholic extract prepared as described previously¹ was blown with a pipette into 5 cc. of saline solution.

TABLE I.

	0.2 cc. emulsion of alcoholic extracts of corpuscles.									
	Blood No. 1	Blood No. 2	Blood No. 3	Blood No. 4	Blood No. 5	Blood No. 6	Blood No. 7	Blood No. 8	Blood No. 9	Blood No. 10
	Group I	Group I	Group I	Group I	Group II	Group II	Group II	Group II	Group III	Group III
0.2 cc. inactivated im- mune sera diluted $\frac{1}{2}$	f. tr.	f. tr.	tr.	+	+++	+++	+++	+++	f. tr.	0
I. S. Group II No. 21	f. tr.	f. tr.	tr.	+	+++	+++	+++	+++	0	0
I. S. Group II No. 22	f. tr.	f. tr.	tr.	+	+++	+++	+++	+++	0	0

TABLE Ia.

	0.2 cc. emulsion of alcoholic extracts of corpuscles.							
	Blood No. 1	Blood No. 3	Blood No. 11	Blood No. 12	Blood No. 6	Blood No. 7	Blood No. 8	Blood No. 13
	Group I	Group I	Group I	Group I	Group II	Group II	Group II	Group II
0.2 cc. inactivated im- mune sera diluted $\frac{1}{2}$								
I. S. Group I No. 17	f. tr.	f. tr.	tr.	0	tr.	tr.	tr.	tr.
I. S. Group I No. 27	0	0	0	0	0	0	0	0

These tests show that with alcohol, group-specific substances can be extracted from erythrocytes. It has been pointed out by Schiff and Adelsberger³ that a similarity exists between a fraction of the group specific part of Group II corpuscles and the heterogenetic antigen of Forssman. We found, however, when emulsions of the two kinds were tested with both Group II immune sera and heterogenetic sera, that the reactions manifested a striking difference provided the emulsions were prepared as described. The alcoholic extract of heterogenetic antigen was made by extracting 1 part of minced horse kidney with 5 parts of 95 per cent alcohol at room temperature for 48 hours. The heterogenetic immune sera were prepared by injections of horse kidney into rabbits.

TABLE II.

0.2 cc. inactivated immune sera diluted $\frac{1}{2}$	0.2 cc. emulsion of horse kidney extract	0.2 cc. emulsion of Group II blood extract
Antihuman II No. 20	tr.	+
Antihuman II No. 21	f. tr.	++±
Antihuman II No. 22	tr.	++±
Heterogenetic No. 402	+++	0
Heterogenetic No. 403	++	0
Heterogenetic No. 54	+++	+

³ Schiff, F., and Adelsberger, L., *Ztschr. f. Immunitätsforsch.*, 1924, xl, 335.

139 (2662)

A study of the biology of streptococcus erysipelatis.**By KONRAD E. BIRKHAUG.** (Introduced by Harold L. Amoss).

[From the Biological Division, Medical Clinic, The Johns Hopkins Hospital, Baltimore, Md.]

The present study was undertaken to determine the immunological reactions of streptococci isolated from erysipelatos lesions. Agglutination and agglutinin absorption were used for biological differentiation. The strains were collected from New York, Washington, and Baltimore, during the summer and fall of 1924. The technique found to be best adapted for the isolation of the streptococci directly from the lesions of erysipelas was as follows: 0.5 cc. of physiological saline solution was injected intra- and sub-dermally at the margin of the erysipelatos lesion. Fluid was withdrawn immediately from the bleb which formed and mixed with blood-agar, according to the method described by Brown.¹ Ninety-one per cent of the cultures from the deeply inflamed marginal lesion yielded *Streptococcus hemolyticus*, whereas cultures made from the central and pale portion of the erysipelatos lesion yielded streptococci only in 42.3 per cent. Culturally and morphologically, these bacteria did not differ in their general characteristics from those of the large group of pyogenic streptococci; all of them were found to ferment lactose and salicin.

For the production of immunebodies, three methods of inoculation of rabbits and dogs were employed: (A) intravenous injection, first with heat-killed and later with live organisms; (B) subcutaneous injection with live bacteria; and (C) Dochez's² method of subcutaneous injection of agar, with subsequent inoculation of live streptococci. The lowest agglutinin titer was obtained with the serum from animals treated by the intravenous inoculation, and the highest and most durable titer by using Dochez's method.

Of the 33 strains of *Streptococcus hemolyticus* isolated from typical erysipelatos lesions, 91.2 per cent were agglutinated by

¹ Brown, J. H., *Monograph of The Rockefeller Inst. f. Med. Research*, 1919, ix, 6.

² Dochez, A. R., *J. Am. Med. Assoc.*, 1924, lxxxii, 542.

the immune sera prepared with seven erysipelalous strains. Of 42 strains of streptococci isolated from non-erysipelalous sources, including scarlet fever, cellulitis, dermatitis, empyema, peritonitis, puerperal sepsis, suppurating tonsillitis, abscesses and mastoiditis, 16.6 per cent were agglutinated by the immune sera prepared with seven erysipelalous strains. The agglutinable non-erysipelalous strains were obtained from the nasal secretions of individuals with chronic and acute sinusitis, and one strain isolated from the meninges of a case of meningitis following mastoiditis. No agglutination occurred with 21 strains of streptococci isolated from throat cultures of scarlet fever patients.

Absorption of agglutinin was best accomplished at 55° C. with fresh immune sera and highly agglutinable strains. Every strain of streptococci of erysipelalous origin was represented in the absorption reactions. The immune serum produced by a given erysipelalous strain was completely absorbed by a number of other erysipelalous strains of streptococci, and the absorption of agglutinin from a number of immune erysipelalous sera was accomplished with each strain.

These investigations demonstrate that at least 90 per cent of the strains of *Streptococcus hemolyticus* isolated from the erysipelalous lesions of erysipelas fall immunologically, as determined by the agglutination and absorption reactions, into one group. This group of hemolytic streptococci can be differentiated, by the same methods, from the type of hemolytic streptococci isolated from patients with scarlet fever.

140 (2663)

Some effects of phosphates parenterally administered.

By IRVINE H. PAGE.

[From the Eli Lilly Research Laboratories, Indianapolis, Indiana.]

Embden, Griesback and Schmitz,¹ Witzemann,² Winter and Smith³ and others seem to have demonstrated that phosphates, both organically and inorganically bound, play an important rôle in carbohydrate metabolism. It thus becomes of interest to know whether inorganic phosphates parenterally administered influence this metabolism as mirrored by blood sugar changes.

Our experiments show that when ortho-phosphoric acid (0.2-2.0 cc. U. S. P. acid in 10 cc. water), mono basic sodium phosphate (1.0-4.0 grams dissolved in the least amount of water), dibasic sodium phosphate (0.5-2.0 grams) and the tribasic sodium phosphate (1.0-3.0 grams) are injected subcutaneously in rabbits (weight 1000-1300 grams) the blood sugar practically always shows a very marked rise. The increased sugar is apparent within twenty minutes, and lasts as a rule for two hours or more. Acid sodium phosphate tends to produce the highest blood sugars when compared with the dibasic and tribasic salt on a weight of salt per kilogram body weight basis; the tribasic is least effective in this respect. The blood sugar rise does not appear to parallel the appearance of the convulsions or tetany to be discussed below, inasmuch as many of the animals showed high blood sugar with no nerve symptoms, and vice versa. Furthermore, a given dose of phosphate in two animals of the same weight does not necessarily produce comparable blood sugar elevations.

Binger⁴ has demonstrated that Na_2HPO_4 , and Na_3PO_4 , given intravenously, produce tetany, but NaH_2PO_4 is inactive in this respect. Tisdall⁵ and Kobert⁶ found H_3PO_4 equally ineffective.

¹ Embden, Griesback and Schmitz, *Zeit. f. Physiol. chem.*, 1914-15, xciii, i.

² Witzemann, *J. Biol. Chem.*, 1920, xlv, i.

³ Winter and Smith, *Brit. Med. J.*, 1923, i, 12.

⁴ Binger, *J. Pharm. Exp. Ther.*, 1917-18, x, 105.

⁵ Tisdall, *J. Biol. Chem.*, 1922, liv, 35.

⁶ Kobert, *Schmidt's Jahrb. d. Ges. Med.*, 1878, clxxix, 225.

Our results confirm these data and further show that NaH_2PO_4 produces marked opisthotonic convulsions. The orthophosphoric acid does not produce convulsions or tetany within the limits in which we have worked—death by respiratory paralysis ordinarily ends the experiment before symptoms become manifest. It would thus appear that either by a change in the $[\text{H}^+]$ or by the addition of the Na^+ or by both we can obtain a wide variation in symptoms (1) sudden death with very few nervous manifestations (H_3PO_4); (2) violent convulsions (NaH_2PO_4); (3) tetany (Na_2HPO_4) and (Na_3PO_4).

141 (2664)

Reflex association of feeding and defecation in young birds. (*Troglodytes Aedon*).

By C. I. REED and B. P. REED.

[From the Hull Physiological Laboratory of the University
of Chicago.]

In connection with the problem of visceral reflexes, it is of interest to record an observation on young house wrens (*Troglodytes aedon*). A brood of six nestlings ranging in age from three to six days was orphaned, and for several hours were fed insects and worms by means of small forceps.

When disturbed, the young bird, if hungry, extended the head and opened its beak. As soon as a morsel of food was swallowed, the bird became very active, scrambling and jostling until it had pushed its head down toward the center of the nest and elevated the anus just over the edge of the nest, in which position defecation always occurred. At once the bird became quiet and could not be induced to take food again for a period of from one and one-half to three minutes. Defecation in this position *always* followed the taking of food.

Ornithologists have long known that in many species the parent birds carried away excreta after every trip to a nest with food. A parent wren was observed to repeat this operation on an average of 25 times an hour, carrying away the excreta *every time* food was carried to the nest.

By noting the color of the food and that of the feces it was made certain that each bolus was digested and the waste ready for voiding before the bird could be stimulated to receive food again. The young bird, then, does not take food until the previous bolus has been digested. Immediately upon swallowing a fresh supply a very complicated reflex is set up, which not only leads to defecation but places the young bird in an unusual position in which the parent bird can most easily collect the excreta as voided and remove it from the nest.

142 (2665)

The rôle of *B. acidophilus* in dental caries.

By R. W. BUNTING and FAITH PALMERLEE. (Introduced by Philip Hadley).

[*From the Pathologic Laboratory of the College of Dental Surgery, University of Michigan, Ann Arbor, Michigan.*]

Since the first pronouncement by Miller¹ in 1890 that tooth caries is the result of the acid fermentation of carbohydrates by bacteria, students of this subject have been searching for a specific organism of caries.

Kligler² first pointed out that in the lesions of caries *B. acidophilus* is commonly present, associated with *L. buccalis*, *C. placoides*, and *B. putrificus*. Later Howe and Hatch³ found in advanced lesions of caries the Moro-Tissier group of organisms associated with certain other types which they called respectively Bacillus X, M, and Y. More recently MacIntosh, James and Lazarus-Barlow^{4, 5} in England, and Rodriguez⁶ in this country, by the use of acid media have found that in deep carious lesions

¹ Miller, W. D., *The Micro-organisms of the Human Mouth*, Philadelphia, 1890.

² Kligler, I. J., *J. Allied Dent. Soc.*, 1915, x, 141, 282, 445.

³ Howe, P., and Hatch, R., *Dental Cosmos*, 1917, lix, 961.

⁴ MacIntosh, J., James, W., and Lazarus-Barlow, P., *Brit. Dent. J.*, 1922, xliii, 728.

⁵ Idem, *Brit. J. Exp. Path.*, 1924, v, 175.

⁶ Rodriguez, F. E., *Military Dental J.*, 1922, v, 199.

B. acidophilus is invariably present and is the most predominant acid-resistant organism associated with the disease. They were also able to produce caries-like lesions in teeth which were immersed in cultures of *B. acidophilus*.

During the past year, in our study of the cause and means of preventing dental caries, we have devoted our attention to a study of the initial stages of caries and the bacteria found to be associated with the first lesion on the tooth. In a study of 73 cases, we found *B. acidophilus* present in 100 per cent of initial caries, in 94 per cent of advanced caries, and in 16 per cent of mouths that were immune to caries. In carious mouths this organism exhibited a high degree of proliferation and a tendency to localize on the tooth, but when present in caries-immune mouths it did not appear to grow in concentrated masses, nor did it predominate in the oral flora as was the case in carious individuals.

We found further that caries-like lesions of the tooth may be produced artificially by lactic acid solutions in concentrations below pH 4.4, in 11 days; also by different strains of *B. acidophilus* in glucose media in from 6 to 8 days when the action was confined to a limited area of the tooth. In a study of the cultural characteristics of over 30 strains of *B. acidophilus*, isolated from carious lesions, it was found that this organism is highly pleomorphic, and that morphologically and biochemically these strains are similar to those isolated from the intestines by Moro.⁷ For this reason we see at the present time no justification for giving this organism a new name such as has been suggested by other workers in this field, nor have we as yet found satisfactory grounds for grouping strains into various types on the basis of morphology and fermentation tests.

Our observation that *B. acidophilus* is intimately associated with the initial lesion of caries, and that this organism is capable of producing caries-like lesions in teeth, *in vitro*, establishes the probability that dental caries is essentially a specific infective process dependent on the presence of certain types of organisms and on environmental conditions favorable to their growth and localized acid formation on the tooth. In the attempt to apply these findings to the clinical control of dental caries, we have combatted overgrowths of *B. acidophilus* on the teeth by direct measures of sterilization. Using a mixed solution of brilliant green and crys-

⁷ Moro, E., *Jahrb. f. Kinderh.*, 1900, lii, 38.

tal violet (1 per cent of each in 50 per cent alcohol), which we have found to be highly antagonistic to *B. acidophilus* (inhibitory in dilutions of 10 to 15 million), we were able to decrease materially the overgrowth of this organism and to improve greatly the hygienic condition of the mouth. From clinical evidence so far obtained it appears that this procedure may be of considerable practical value in the control of dental caries.

143 (2666)

Continuous recording changes in hydrogen ion concentration of circulating blood: The relation to respiration.

By ROBERT GESELL and ALRICK B. HERTZMAN.

[*From the Department of Physiology, University of Michigan, Ann Arbor, Michigan.*]

In the study of the chemical regulation of respiration a need for a continuous method of recording changes in the hydrogen ion concentration of the circulating arterial and venous blood was felt. Such a method has been developed.

By means of a specially devised electrode vessel, a manganese dioxide electrode was placed in the circulating blood. The chain was closed with a non-polarizable electrode, and the E. M. F. recorded potentiometrically on smoked paper by means of a writing point attached to the hard rubber drum of a Leeds and Northrup type K potentiometer.

The continuity of the method, the facility of recording changes in C_{H^+} , the amount of data obtainable from single animals, and the possibility of recording synchronous changes in C_{H^+} in the arterial and venous blood along with changes in pulmonary ventilation, oxygen consumption, blood pressure, etc., are advantages which make the method extremely valuable. In experiments so far performed the method has shown characteristic changes in the C_{H^+} of the blood with various procedures.

The administration of CO_2 eliciting increased pulmonary ventilation was accompanied by a sharp rise in the C_{H^+} of the arterial blood, followed by a slower fall in C_{H^+} during recovery.

The intravenous injection of NaHCO_3 produced a sudden fall in the C_{H^+} of the arterial blood, followed by a slow return to normal. Though the changes in C_{H^+} were large they were unaccompanied by changes in pulmonary ventilation. See Figure 1.

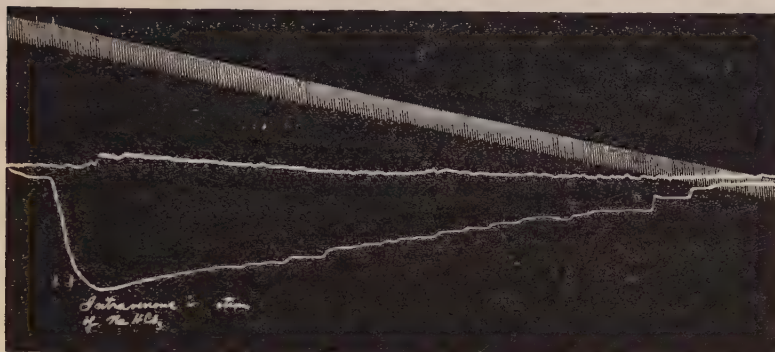


FIG. 1.

Occlusion and de-occlusion of the trachea produced typical changes in blood pressure and respiration. The C_{H^+} record resembled in detail the form of the blood pressure record. A record of such an experiment is shown in Figure 2.

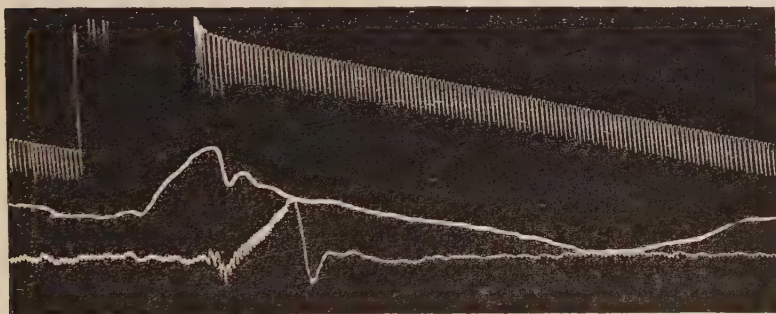


FIG. 2.

The administration of rarefied air eliciting increased pulmonary ventilation was accompanied by a decrease in the C_{H^+} of the arterial blood. Subsequent administration of room air was followed by a further short but sudden decrease in C_{H^+} , giving way to an increased C_{H^+} .

Spontaneously occurring periodic respiration associated with

periodic changes in blood pressure was accompanied by periodic changes in the C_{H+} of the arterial blood. See Figure 3.

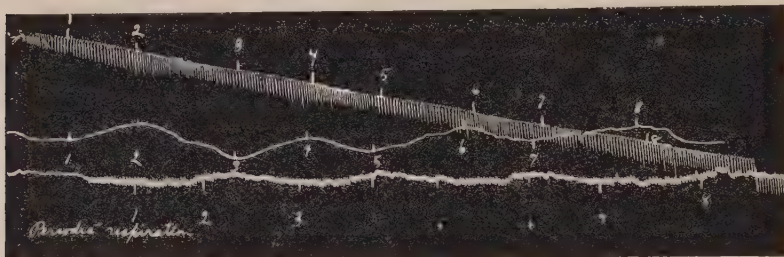


FIG. 3.

The intravenous injection of NaCN produced a decrease, an increase, a decrease and a final increase in the C_{H+} of the arterial blood associated respectively with an increase, a decrease, an increase and a decrease in pulmonary ventilation.

Moderate hemorrhage elicited a decreased C_{H+} accompanied by an increased pulmonary ventilation. Subsequent injection of gum-saline solution decreased pulmonary ventilation and increased the C_{H+} of the blood.

Severe hemorrhage elicited a primary decrease in C_{H+} which suddenly gave way to an increasing C_{H+} . Injection of gum-saline solution during the increasing C_{H+} resulted in a decreased pulmonary ventilation and a decrease in C_{H+} to normal values.

Though the injection of gum-saline solution following severe hemorrhage eventually led to a lowering of the C_{H+} of the blood, it was followed for a minute or more by an increasing C_{H+} produced by the preceding anemia. Such an increasing C_{H+} was associated with a reduction, almost a cessation of pulmonary ventilation. It seems highly probable that the tissues were turning alkaline while the blood was still turning acid.

Simultaneous records of C_{H+} changes in the arterial and venous blood showed more abrupt but longer and greater changes in the arterial than in the venous blood.

The results uphold in general our view that there is no constant relation between the composition of the blood and pulmonary ventilation; that the metabolism of the respiratory center itself is an important factor controlling pulmonary ventilation. This is indicated by changes in pulmonary ventilation, accompanying changes in the volume flow of blood independent of change or direction of change in the composition of the blood.

144 (2667)

Further note on the fusus coli of the rabbit.

By JOHN AUER.

[From the Department of Pharmacology of St. Louis University
School of Medicine, St. Louis, Missouri.]

In a preceding communication an apparently unknown spindle-shaped structure in the rabbit was described, which connects the transverse colon with the descending colon. Evidence was submitted which proved that this section of the gut forming the spindle was anatomically and functionally different from the neighboring transverse and descending colon. It was shown in brief that this organ was slightly curved, spindle shaped, 4.5 to 8.5 cm. in length along the greater curvature, provided with a sphincter towards the descending colon side, devoid practically of any mesentery, more pink and thicker-walled than either the ascending or the transverse colon, with a thick, smooth mucosa arranged in longitudinal folds, and quite different from that found in adjoining sections of the gut. It was emphasized that the scybala found in normal rabbits towards the ascending colon side were large, grayish, soft and rich in water, while those immediately below the sphincter in the descending colon, were smaller, grayish yellow, hard and dry.

Functionally the organ behaved as follows in a morphinized rabbit after laparotomy: the intravenous injection of 0.1 to 0.3 mg. of physostigmin caused well marked peristalsis in the ascending and transverse colon. As the wave of contraction reached approximately the middle part of the spindle, the neck of the spindle contracted powerfully and antiperistaltically, preventing the passage of the scybalum. The *peristaltic* wave relaxed first, and the contents of the bulging portion between the two constrictions is pushed back *antiperistaltically* by the maintained or increasing antiperistaltic contraction of the sphincter area. After this the sphincter area relaxes slowly. This phenomenon is repeated several times, and occasionally gas and liquid can be seen passing through the narrowing sphincter. Finally, however, the antiperistaltic contraction wave at the neck relaxes before the peristaltic contraction advancing from the transverse colon, and then the contents of the spindle is pushed into the dilating thin-

walled descending colon. When now 0.2 to 0.5 cc. adrenalin are injected intravenously, the spindle exhibits more or less powerful rhythmic contractions and relaxations while the rest of the gut, especially the descending colon and small intestine, are largely motionless and relaxed.

Stimulation of the peripheral stumps of the vagi (sectioned beneath the diaphragm) or of one splanchnic nerve (cut below the diaphragm) cause motor responses like those described after physostigmin and adrenalin. Inhibitory effects were also observed, both after nerve stimulation and after drugs.

Still more evidence that the *fusoid coli* is a morphological entity is revealed when this structure is studied microscopically. The spindle as well as portions of the transverse colon, ascending colon, caecum and duodenum were excised from a freshly killed, normal rabbit, washed clean in saline or Ringer solution, then filled with Orth's fluid, ligated, and immersed in liberal amounts of the same fixative. After fixation, the tissue was prepared for mounting in paraffin, and longitudinal and transverse sections were cut and stained with haematoxylin-eosin and Van Giesen.²

The main results are as follows:

Longitudinal muscle layer:

At the neck of the spindle, the site of the sphincter, this coat forms a continuous layer enveloping the entire circumference; it varies in thickness in different places of the same transverse section, but is always definitely thicker than in the descending colon or in the transverse colon.

Circular muscle coat:

This layer is markedly thickened at the spindle neck, forming a definite sphincter, and may be more than four times as thick (viz. 140 m.) as the same layer in the ascending and transverse colon, and descending colon. This layer then gradually thins as it approaches the transverse colon.

Mucosa:

In the spindle-body this layer may be 4 to 5 times thicker than the mucosa of the ascending colon, for example 630 m. against 120 m. It exhibits no papillæ like the ascending and transverse colon, but is composed of closely packed, simple tubular glands,

² Thanks are due to my colleague, Dr. Pohlman, for kindly permitting his technician to carry out this work.

standing at right angles to the *muscularis mucosæ*, which they do not perforate. The neck of these glands shows mucus cells.

The plexus of Auerbach at the neck of the spindle discloses thicker and apparently more numerous ganglion cell-aggregates than are generally found in the transverse and ascending colon.

145 (2668)

Continuation of secretion of the ovarian follicular hormone by the human corpus luteum.

By EDGAR ALLEN and EDWARD A. DOISY.

[From the Department of Anatomy, University of Missouri, and the Laboratories of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Mo.]

Our studies have demonstrated the presence of a hormone in the ovarian follicles of hens, swine, cattle, sheep and women.^{1,2,3,4} These tests seem sufficient to indicate the expected non-specificity of this substance among different species. Repeated tests of similarly prepared extracts of *corpora lutea* of both oestrous and pregnancy from swine and cattle have shown that this hormone is not present in appreciable amounts in the fully formed corpora of these animals. These data seemed to warrant the general conclusion that the ovarian follicle produces the stimulus which periodically causes growth and secretion in the tissues of the genital tract, and that this function wanes rapidly or is lost after ovulation.

Since our earlier interpretations were made we have had an opportunity to extend our work to tests of human ovarian tissues, chiefly through the interest and co-operation of Doctor J. P. Pratt of the Henry Ford Hospital, Detroit. The results of these experiments, which are tabulated below, seem to indicate that the human *corpus luteum*, unlike that of the sow and the cow, continues the secretion of the follicular hormone for an appreciable period.

¹ *Am. J. Anat.*, 1924, xxxiv, 133.

² *J. Biol. Chem.*, 1924, lxi, 711.

³ *Am. J. Physiol.*, 1924, lxix, 577.

⁴ *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 500.

TABLE 1.

Tests of human ovarian tissues for the presence of the follicular hormone.

Extract	Specimen	History	Volume	Results
IL	Liquor folliculi	Normal follicles (medium sized)	3.0 cc.	+
XL	Liquor folliculi (6 different samples)	Cystic follicles	5-30 cc.	+ (10 tests)
5C	Corpus luteum	2 wks. post menstrum	1.4 cc.	+
6C	Corpus luteum	3 wks. post menstrum	5.0 cc.	+
7C	Corpus luteum	3 wks. post menstrum	2.6 cc.	+
8C	Several corpora	(one corpus, after recent ovulation)	5.0- cc.	+
9C	Corpus luteum	25 dys. post menstrum	2.0 cc.	—
10C	Corpus luteum	No history	2.0 cc.	+
11C	Corpus luteum	1st month of pregnancy ⁵		+
13C	Corpus luteum	2nd month of pregnancy	1.0 cc.	±
14C	Corpus luteum	3rd month of pregnancy	1.6 cc.	+

The material tested includes fluid aspirated from medium sized normal follicles and large follicular cysts,⁶ and *corpora lutea* enucleated from the ovary at known intervals after the preceding menstrual period (three of these were corpora of early pregnancy). All tissues tested were removed at operation. The corpora were enucleated with very little adherent stroma, and after extraction sections were made for histological study. The liquor folliculi does not contain the total amount of hormone present in the follicle, for additional amounts may be extracted from the follicle cells lining the walls which are not removed by aspiration of the other follicular contents.

Extracts were made with lipid solvents according to the method described for the preparation of the follicular hormone.^{2, 4} It will be noted that in some cases the tissue extracted was less than 2 cc. in volume.

These extracts were tested (in some cases quantitatively) by injections into ovariectomized rats, as previously described.¹ A positive test means the induction of maximum oestrous growth

⁵ Lipoid extracts of placenta (3 and 7 months and full term) and of two chorionic vesicles (6 weeks and 2 months) have also returned positive results.

⁶ For three of these specimens we are indebted to Doctors Q. U. Newell and F. P. McNally of Washington University School of Medicine, St. Louis, Mo.

in the vaginal epithelium, which amounts to the addition of from 10 to 16 new layers of cells in 48 hours, and results in a cornification process in this tissue. This rapid growth in the vagina is correlated with a corresponding growth and secretion in the uterus.

Therefore, an active extract substitutes for this ovarian secretion instead of merely influencing the growth and secretion of the intact ovaries. This is the point we wished to make in attempting a distinction between this hormone as the "causative" mechanism and other possible "regulatory" factors in the growth changes in the female genital tract.⁷

146 (2669)

Observations on intravital staining of centrifuged marine eggs.

By BALDWIN LUCKÉ.

[From the Laboratories of the Bureau of Fisheries, Woods Hole, Mass., and the Pathological Laboratory, School of Medicine, University of Pennsylvania, Philadelphia, Pa.]

That living protoplasm cannot be stained has long been known but is not as yet generally appreciated. Because of the numerous cellular inclusions (granules, globules and vacuoles of various kinds) which do take up the dye, a cell stained *intravital* may appear stained as a whole, but upon separating the inclusions from the protoplasmic matrix the latter will be found free from the dye. The relatively large eggs of certain marine invertebrates furnish excellent objects for demonstrating this fact, since by centrifugation the formed elements may be separated from the protoplasmic ground substance. If *Arbacia* eggs are centrifuged, the cell contents separate into four well-defined zones; the lipid globules are massed at one pole, the pigment granules at the opposite pole; adjoining the pigment zone is a layer of granules of varying sizes, and between this and the mass of lipid globules, a band of optically empty, homogenous cytoplasm. The width of these four zones depends, to a certain extent, upon the

⁷ *Am. J. Anat.*, 1924, xxxiv, 161, 164.

length of time the eggs have been subjugated to centrifugal force. In the egg of the clam, *Cumingia*, only three zones are formed (the pigment and granular zones do not separate).

Freshly obtained unfertilized *Arbacia* eggs were centrifuged in small haematocrit tubes; the zoned eggs were then placed in a 1:40,000 solution of neutral red or brilliant cresyl-blue in seawater. It was found that only the pigment and granular zones took up the dye; the clear cytoplasm and the lipoids remained unstained. Identical results were obtained when the eggs were first stained and then centrifuged. Examination with dark field illumination showed the zoning with particular clearness, and brought out the neutral red stained granules with great brilliance. *Arbacia* eggs stained and centrifuged could be fertilized, and many developed to the gastrula stage.

An apparent exception to the general observation that living cytoplasm does not stain was found to occur when the centrifuged eggs were exposed to the dye for so long a time that the cytoplasm became very finely granular, that is, underwent a granular degeneration. The apparent staining of the protoplasm was probably due to coloration of the innumerable, closely packed, extremely minute granules in the injured protoplasm.

In an effort to determine the reaction of the protoplasm and its various inclusions, *Arbacia* or *Cumingia* eggs were placed in weak solutions of dibromthymolsulphonaphthalein (brom thymol blue)¹ in seawater. The indicator penetrated slowly but many eggs were eventually colored a deep yellow; however, on centrifuging them they failed to separate into layers, indicating that they had been killed, and that no information was afforded as to the reaction of the cell while living.

These experiments constitute further evidence and a ready method for demonstration that living protoplasm cannot be stained by such relatively non-toxic dyes as neutral red or brilliant cresyl-blue; the method also furnishes means for determining whether an alleged vital dye has injured or killed the cell by coagulating the cytoplasm.

¹ Crozier had found this compound non-toxic for such cells as *Paramecium* and *Opalina* whose cytoplasm stained diffusely yellow, thereby indicating an acid reaction. PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 58.

THE MINNESOTA BRANCH.

University of Minnesota, January 7, 1925.

147 (2670)

A quantitative study of the physiologic action of thyroxin.

By EDWARD C. KENDALL.

*[From the Mayo Foundation, University of Minnesota,
Rochester, Minn.]*

The isolation of thyroxin in pure crystalline form permitted the fact to be shown that thyroxin alone increases the rate of combustion in the animal organism. Furthermore, this increase is related quantitatively to the amount injected. One milligram given to an adult produces an increase of approximately 2.5 per cent. The substance acts in minute amounts for long periods, and produces such enormous increase in the output of carbon dioxid above the former level, that there is no escape from the conclusion that thyroxin acts as a catalyst.

Through a study of closely related compounds, which were synthetically prepared, the fact was demonstrated that thyroxin can exist in two forms: reduced and oxidized. Thyroxin, as isolated from the gland, is the reduced form.

Alpha oxy indol propionic acid, the precursor of thyroxin, acts as a reducing agent. It loses two atoms of hydrogen, with molecular oxygen, when the pyrrol ring in the molecule is open, and forms a bond from the nitrogen, to the number seven carbon in the benzene ring. This compound has feeble oxidizing power. When, however, the pyrrol ring is closed and the bond is present from the nitrogen to number seven carbon, the oxidizing power of the compound is very much increased. The oxidizing potentials of the open and the closed ring compounds, when both exist in their oxidizing form, have been measured, and a difference of at least 0.3 volt was found.

When they are injected into a normal dog, the reduced form, and the oxidized but open ring form, produce no visible response; the oxidized closed ring form causes a marked physiologic effect. There is a drop in blood pressure, an increase in pulse rate, a marked increase in respiration, and an increase in the rate of metabolism.

It is significant that oxidation in the animal organism is accelerated by the presence of an agent which is an active hydrogen acceptor, and the degree of stimulation is dependent on the oxidizing potential of this hydrogen acceptor.

The function of thyroxin is to furnish a compound that can be acted on by mild oxidizing agents, among which is molecular oxygen, and which can then by an intramolecular re-arrangement produce an intensely oxidizing substance.

This same mechanism of increasing the intensity of oxidation is evidently a reaction which is used by other catalytic agents in the body, bringing about an increased rate of combustion.

148 (2671)

The mechanism and significance of the fragility test.

By R. G. GREEN.

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.*]

If erythrocytes are treated with a solution of castor oil soap of such concentration that the liberation of hemoglobin is complete in about ten hours, there is a period of several hours before any hemolysis takes place. Fragility tests during this period show that there is a decreased fragility of these cells to hypotonic salt solution.¹ As these cells differ from normal cells in that they are being subjected to an accelerated hemolysis, the decreased fragility indicates an injury to the cell.

It has been well established that upon injury or death, there is an exosmosis of salts from cells. The work of G. N. Stewart² has shown that blood cells may lose salts by exosmosis without the liberation of hemoglobin. It would appear then that when blood cells are immersed in a hypotonic salt solution, not only does water pass into the cells, but salts also pass out. The most dilute salt solution in which blood cells will not hemolyze, represents a situation in which enough salts can pass out of the cell,

¹ Green and Evans, *Proc. Soc. Exp. Biol. and Med.*, 1923, xv, 290-291.

² Stewart, G. N., *J. Pharmacol. and Exp. Therap.*, 1910, i, 49.

and bring about osmotic equilibrium, before sufficient water can pass in to liberate the hemoglobin. This is indicated by the following: Normal red blood cells transferred successively to solutions of lower salt concentration may be finally introduced into a 0.3 per cent NaCl solution without liberation of hemoglobin.

A decreased fragility of erythrocytes, then, represents not a greater strength but an inability to maintain an osmotic difference from the surrounding solution because of greater permeability of the cell wall to the contained salts. That soap-treated cells have an increased permeability coördinated with a decreased fragility, is shown by successive transfer to more dilute salt solutions, when the cells will not liberate hemoglobin in 0.15 NaCl solution. Electrical resistance measurements of cells treated with castor oil soap, measurements made with H. O. Halvorson, and the specific resistances calculated with MacDougall's formula for disperse systems,³ have given values about half as great as those found by MacDougall and Green⁴ for normal cells (2000 ohms). This is another indication of the increased permeability of cells exhibiting decreased fragility.

The above mechanism of the fragility test based on experimental results allows a satisfactory explanation of the decreased fragility in hypotonic salt solution found typically in the case of pernicious anemia. The blood cells in pernicious anemia are injured cells and are being subjected to an accelerated hemolysis.

149 (2672)

The fragility of human erythrocytes after treatment with pernicious anemia serums.

By R. G. GREEN.

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota.*]

It has been previously reported from these laboratories¹ that the resistance of normal cells to hemolysis by hypotonic saline solution is greatly increased by treating the normal cells with

³ MacDougall, F. H., *Science*, 1924, lix, 403.

⁴ MacDougall and Green, *J. Infect. Dis.*, in press.

¹ Green, *Proc. Soc. Exp. Biol. and Med.*, 1923, xv, 291-292.

serum from a pernicious anemia patient whose erythrocytes show an increased resistance with the same test. Further experiments in which the cells were treated with various dilutions of the serum made with physiologic salt solution show the effect to be marked when the serum is diluted 1 to 500, sometimes appreciable when diluted 1 to 1000, but usually absent when diluted 1 to 2000. The activity of the serum varied in this respect in different cases. The results of a typical experiment are given below. All cells were washed in salt solution before performing the test.

EXPERIMENTAL DATA.

CHART 1.

Concentration of NaCl	Hemolysis time for patient's cells (pernicious anemia)	Normal cells	Normal cells treated with pernicious anemia serum.					
			Serum undiluted	Serum diluted 1:10	Serum diluted 1:100	Serum diluted 1:500	Serum diluted 1:1000	Serum diluted 1:2000
0.30%	20.2 sec.	12.4 sec.	17.4 sec.	64.6 sec.	34.3 sec.	21.6 sec.	19.2 sec.	12.8 sec.
0.35%	Trace in 3 hrs.	17.6 sec.	25.4 sec.	Trace in 3 hrs.	Partial hemolysis in 3 hours.	3600 sec.	120 sec.	17.2 sec.
0.40%	No hemolysis	48.8 sec.	No hemolysis	No hemolysis	No hemolysis	No hemolysis	300 sec.	43.4 sec.
0.45%	No hemolysis	No hemolysis	No hemolysis	No hemolysis	No hemolysis	No hemolysis	No hemolysis	No hemolysis

CHART 2.

Concentration of NaCl	Patient's cells	Normal cells	Normal cells treated with pernicious anemia serum.					
			Serum undiluted	Serum diluted 1:10	Serum diluted 1:100	Serum diluted 1:500	Serum diluted 1:1000	Serum diluted 1:2000
0.30%	48.4 sec.	13.4 sec.	36.2 sec.	31.6 sec.	19.4 sec.	13.6 sec.	13.2 sec.	12.8 sec.
0.35%	109.8 sec.	17.4 sec.	48.3 sec.	43.2 sec.	38.2 sec.	18.5 sec.	18.3 sec.	18.9 sec.
0.40%	No hemolysis	39.6 sec.	122.5 sec.	68.0 sec.	52.6 sec.	40.2 sec.	39.0 sec.	40.2 sec.
0.45%	No hemolysis	360 sec.	No hemolysis	Trace in 3 hrs.	543 sec.	398 sec.	382 sec.	335 sec.

These experiments indicate that there is some agent present in the blood serum of an individual with pernicious anemia which affects the red cells to give them the characteristic decreased fragility to hypotonic salt solution, and this agent may be present in an amount sufficient to have an appreciable effect when the serum is diluted a thousand times.

In an earlier paper it was reported² that blood cells treated with a weak solution of castor oil soap showed, before the liberation of hemoglobin, the decreased fragility to hypotonic salt solution characteristic of pernicious anemia. This would indicate that the substance in pernicious anemia serum which brings about the decrease in fragility of normal cells when immersed in it, may be the hemolytic agent responsible for the anemia.

150 (2673)

The effect of quinidine on interauricular conduction and irritability in the terrapin's heart.

By ARTHUR D. HIRSCHFELDER and CHARLES CERVENKA.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minn.*]

Numerous investigators have demonstrated that quinidine decreases the irritability of the heart muscle and the auriculo ventricular conduction. We have recorded the contractions of both auricles in the terrapin, and find that, although much stronger stimuli (rhythmic make-and-break shocks) are required to cause extrasystolic responses after intraventricular injections of 1-2 mg. quinidine sulphate than before, there is no marked disturbance of conduction of spontaneous contractions or of rapid rhythmic extrasystoles from right auricle to left auricle. This indicates that quinidine depresses irritability more than it depresses intra- and inter-auricular conductivity; and renders it probable that in auricular fibrillation it rather suppresses the genesis of ectopic impulses than that it blocks the conduction of circus move-

² Green and Evans, PROC. SOC. EXP. BIOL. AND MED., 1923, xv, 290-291.

ments when once generated. Our results are in accord with the investigations of Hirschfelder¹ in 1908, which indicated that increased irritability of the heart muscle is one of the important factors in the genesis of auricular fibrillation.

151 (2674)

The effect of pituitrin on blood and on lymph and urine production.

By E. C. BAYLEY, J. C. DAVIS, W. WHITMAN, and F. H. SCOTT.

[From the Department of Physcology, University of Minnesota, Minneapolis, Minn.]

The diuretic action of pituitary extract described first by Magnus and Schäfer¹ has since been observed by many. More recently an anti-diuretic action of this extract has been observed, especially in connection with *diabetes insipidus*, and in relation to water intoxication. (For literature see Weir, Larson and Rowntree.²) On account of its diuretic action one might expect some effect on the composition of the blood. Underhill and Pack³ and Mackersie⁴ observed a dilution of the blood after pituitrin injection, but only a few experiments were made. Rowntree,² however, observed no change in blood volume, and Lamson,⁵ who injected physiological salt along with pituitrin observed no change with small doses, but found that after large doses of pituitrin, the physiological salt did not leave the blood as it normally does, but kept the blood diluted for hours.

We have followed the concentration of the blood in 20 dogs after pituitrin injection, using 2 or 3 cc. of Parke Davis's ob-

¹ Hirschfelder, A. D., *Bull. Johns Hopkins Hospital*, 1908, xxix.

² Magnus, R., and Schäfer, E. A., *J. Physiol.*, 1901, xxvii, 9.

³ Weir, J. F., Larson, E. E., and Rowntree, L. G., *Arch. Int. Med.*, 1922, xxix, 306.

⁴ Underhill, F. P., and Pack, G. T., *Am. J. Physiol.*, 1923, lxvi, 520.

⁵ Mackersie, W. G., *J. Pharmacol. and Exp. Therap.*, 1924, xxiv, 83.

⁶ Lamson, P. D., Abt, A. F., O. Osthuisen and Rosenthal, S. M., *J. Pharmacol. and Exp. Therap.*, 1923, xxi, 401.

stretical pituitrin. In 16 of these cases the effect was so very slight as to be within the limit of error. Most of the slight effects observed tended to be more toward dilution than towards concentration. In one case there was a marked dilution of the blood; the hemoglobin falling to 74.1 about an hour after injection, corresponding to the effect observed by Underhill. In three cases there was a marked increase in the concentration of the blood, the hemoglobin rising to 111, 108 and 107, respectively. A concentration of the blood is what one might expect⁶ from a rise of blood pressure. We have also confirmed Lamson's observations that physiological salt is delayed in its exit from the vessels.

The flow of lymph from the thoracic duct has been followed in 11 cases, and in 9 of these the urine flow was also studied. In every case the injection of pituitrin caused a marked slowing of the lymph flow which lasted some time. One example may be given from a dog anaesthetized with morphia and ether: normal from the thoracic duct, 3 drops per minute; injection of physiological saline (300 cc.) caused an increase to 13 drops per minute; injection of 2 cc. pituitrin caused a drop to 6 cc. per minute which remained at that level for one hour, when a further injection of physiological saline (200 cc.) caused an increase to 23 drops per minute. A second injection of pituitrin reduced it to 9 drops in three minutes, and to 3 drops, 15 minutes later, when the flow gradually increased to 12 drops. A third injection of pituitrin reduced it again to 6 drops.

Another example showing the relation of lymph and urine flow may be given. These are drops per minute for the first ten minutes after, and the 15th and 20th minute.

Lymph	3	4		7	4	1	0	1	1	1	1	2	2.....3.....7
Urine	16	15		11	7	3	29	48	34	45	43	42	38.....24.....19

x

At x injection of 2 cc. pituitrin.

The remarkable manner in which the lymph flow is cut down by pituitrin should be noted, and also the temporary slowing down of urine flow. This was also observed by Schäfer and Magnus. We believe the results on lymph flow and the results on the manner in which pituitrin keeps physiological saline in the blood, as well as its effect in *diabetes insipidus* and water intoxication, can only be explained on the basis of its rendering the capillaries less per-

⁶ Scott, F. H., *Am. J. Physiol.*, 1917, xliv, 298.

meable. Even the cells of the kidney seem to be rendered less permeable, but the increased blood pressure overcomes this effect and diuresis follows. The diuretic action of pituitrin probably depends on the vascular changes in the kidney (Knowlton and Silverman⁷) while its anti-diuretic action depends on the lessened permeability of the cells. The dilution of blood following pituitrin is, we believe, due to the absorption of fluid from the intestinal tract and the subsequent holding of the fluid in the blood.

⁷ Knowlton, F. P., and Silverman, A. C., *Am. J. Physiol.*, 1918, xlvii, 1.

SCIENTIFIC PROCEEDINGS.

NEW YORK MEETING.

*University and Bellevue Hospital Medical College, New York
City, March 18, 1925.*

152 (2675)

Some factors affecting the levels of the serum calcium and
phosphorus of normal rabbits.

By J. H. B. GRANT and FREDERICK L. GATES.

*[From the Rockefeller Institute for Medical Research, New York
City.]*

At intervals throughout the past year, with the exception of the summer months, we had occasion to make a considerable number of determinations of the inorganic calcium and phosphorus content of normal rabbits' sera. The results are of interest in that they reveal certain progressive variations due apparently to seasonal changes in the environment, and they also show an effect of caging that should be taken into account.

As received from various dealers, 144 male rabbits showed a blood calcium level of 12.31 mg. per 100 cc. of serum.¹ This general average was obtained, however, from figures that varied considerably for different months of the year, (Table I.) From a low level of 11.74 mg. in January, high levels were reached in May and November of 12.92 and 12.88 mg. Similar variations were found in the blood calcium level of 82 normal rabbits (110 determinations) which had been caged, individually, indoors in a well lighted and ventilated room, and fed hay, oats and cabbage for periods of 1 to 7 weeks. The figures for these rabbits parallel those obtained on admission, but show the blood calcium to be

¹ Method of Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvii, 475.

TABLE I.

Month	Calcium*		Phosphorus*	
	On Admission	After Caging	On Admission	After Caging
1924				
Jan.	11.74 \pm 0.11	12.40 \pm 0.11	6.35 \pm 0.20	6.24 \pm 0.62
Feb.	-----	13.15 \pm 0.18	-----	-----
March	12.61 \pm 0.13	13.32 \pm 0.33	7.44 \pm 0.69	7.69 \pm 0.40
April	12.64 \pm 0.16	13.20 \pm 0.12	6.97 \pm 0.31	6.71 \pm 0.21
May	12.92 \pm 0.26	13.62 \pm 0.12	4.22 \pm 0.24	6.60 \pm 0.25
June	-----	13.53 \pm 0.25	-----	5.51 \pm 0.31
Sept.	12.35 \pm 0.08	12.44 \pm 0.10	7.03 \pm 0.16	7.08 \pm 0.44
Oct.	12.42 \pm 0.12	12.92 \pm 0.23	5.31 \pm 0.15	7.45 \pm 0.15
Nov.	12.88 \pm 0.14	13.47 \pm 0.16	5.83 \pm 0.15	6.24 \pm 0.20
Dec.	12.31 \pm 0.17	-----	6.26 \pm 0.33	-----

*In milligrams per 100 cc. blood serum. Probable errors calculated by Peters' formula.

at a uniformly higher level—13.12 mg. per 100 cc. serum—with the January low level at 12.40 mg., and the May and November peaks at 13.62 and 13.47 mg. respectively. We have already noted² that the caging of normal rabbits, under conditions that obtain with us, results in a definite rise in the blood calcium. It remains to be seen how the apparent seasonal variations in the blood calcium can be correlated with the changes in the size and functional activity of certain elements in the endocrine system which Drs. Brown, Pearce and Van Allen have observed at the same seasons of the year.³

Fifty-five of the normal rabbits were examined on admission and at intervals thereafter up to a period of 7 weeks. In general, the longer the animals were caged, the higher the blood calcium rose, showing an increase from 12.52 mg. to 13.65 mg. per 100 cc. serum during this period (Table II). These animals served as controls for successive groups of rabbits irradiated daily with a quartz mercury arc lamp over corresponding periods of time. The irradiated rabbits showed a similar increase in blood calcium during the first four weeks of exposure—the period of progressive parathyroid hypertrophy under the influence of ultra-violet light.² Coincident with the subsequent drop in the size of the parathyroid glands that occurred in those rabbits that were irradiated for five to seven weeks, the five-week group showed a

² Grant, J. H. P., and Gates, F. L., *J. Gen. Physiol.*, 1924, vi, 635.

³ Brown, W. H., Pearce, L., and Van Allen, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 373.

FACTORS AFFECTING SERUM CALCIUM AND PHOSPHORUS 317

TABLE II.

Days	Calcium*		Phosphorus*	
	Caged	Radiated	Caged	Radiated
0-6	12.52 \pm 0.11	12.33 \pm 0.05	6.56 \pm 0.18	6.20 \pm 0.17
7-13	12.56 \pm 0.25	12.88 \pm 0.18	7.24 \pm 0.22	5.83 \pm 0.21
14-20	13.25 \pm 0.11	12.95 \pm 0.20	6.10 \pm 0.14	7.21 \pm 0.33
21-27	12.93 \pm 0.13	13.00 \pm 0.13	6.41 \pm 0.25	8.19 \pm 0.27
28-34	13.31 \pm 0.13	12.48 \pm 0.09	6.89 \pm 0.51	7.29 \pm 0.24
35-41	13.40 \pm 0.21	12.86 \pm 0.21	-----	7.23 \pm 0.27
42-49	13.65 \pm 0.20	13.05 \pm 0.12	-----	5.74 \pm 0.21

*In milligrams per 100 cc. blood serum. Probable errors calculated by Peters' formula.

drop in blood calcium to the level for the whole group on admission. The groups radiated for six or seven weeks gave somewhat higher figures. These observations are in line with our suggestion that, while parathyroid activity is necessary to the maintenance of the calcium level, other independent factors, as yet not understood, determine the upper limit of calcium concentration in the blood.

In the tables we have recorded the observations on inorganic phosphorus⁴ in most of the specimens of blood used for the calcium determinations. Hess and Lundagen⁵ have observed seasonal variations in the blood phosphorus of infants, with a low level in March. So little is known of the causes and significance of changes in the blood phosphorus level that we present these figures only for record, and without an attempt at interpretation. It may be noted, however, that, in general, between March and December the higher calcium levels were accompanied by lower phosphorus levels in the blood, and *vice versa*. Howland and Kramer⁶ have observed that in tetany a rise in calcium may be accompanied by a corresponding fall in phosphorus, so that the product of their concentrations may be actually decreased. On the other hand, the figures in Table II show that in the irradiated rabbits the highest levels of both phosphorus and calcium coincided with the period of most marked parathyroid hypertrophy, and that both elements in the blood fell during the following period when the glands were undergoing regression.

⁴ Method of Tisdall, F. F., *J. Biol. Chem.*, 1922, ii, 329.

⁵ Hess, A. F., and Lundagen, M. A., *J. Am. Med. Assn.*, 1922, lxxix, 2210.

⁶ Howland, J., and Kramer, B., *Monatsch. fr. Kinderheilk.*, 1923, xxv, 279.

153 (2676)

L. Acidophilus versus L. Bulgaricus milk feeding.

By NICHOLAS KOPELOFF and PHILIP BEERMAN.

[From the Department of Bacteriology, Psychiatric Institute,
Ward's Island, New York City.]

Experiments of this kind have been carried out on groups of four human subjects by Rahe,¹ and by Rettger and Cheplin.² Because of its importance, 12 psychotic subjects showing on repeated fecal examination no *acidophilus-bulgaricus* type of colony were fed 3 strains of *L. bulgaricus* milk for 14 days, after a preliminary observation period of 14 days. *L. bulgaricus* was not recovered from the feces, except in one instance.

After an interval of 14 days, *L. acidophilus* milk was fed for 14 days. The constipated cases were greatly benefited, and the non-constipated cases also had a greater number of normal defecations. *L. acidophilus* was recovered from the feces of all subjects. In three cases the per cent was 99-100, and in all cases but one, it was over 67.

Subsequently, some cases continued to show marked improvement over their original condition, while others showed a tendency to revert to it. Seven days after discontinuing treatment, there were no *L. acidophilus* present in the feces.

It is thus clearly established that *L. acidophilus* differs from *L. bulgaricus* in its ability to live in the gastro-intestinal tract. In a further inquiry into the nature of the activity of *L. acidophilus*,³ we are studying the enzymes of this organism, and have good reason to believe that they differ from those of *L. bulgaricus*. Another interesting chemical problem being considered is the stereoisomerism of the lactic acid produced by these Lactobacilli.

¹ Rahe, A. H., *J. Infect. Dis.*, 1915, xvi, 210-220.

² Rettger, L. F., and Cheplin, H. A., *The Intestinal Flora*, Yale University Press, 1921.

³ Kopeloff, N., and Beerman, P., *Arch. Int. Med.*, 1924, xxxiii, 55-57.

154 (2677)

Some properties of cholesterol and phytosterol activated by irradiation.

By ALFRED F. HESS and MILDRED WEINSTOCK.

[From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.]

In a previous communication¹ it has been shown that cholesterol and phytosterol can be activated by means of ultra-violet irradiation so as to acquire protective value against rickets. Further experiments have demonstrated that irradiated cholesterol also evinces this potency when given by the subcutaneous route. By employing selective filters it was ascertained that rays of approximately the same wave lengths were effective in activating cholesterol as had previously been found of value in protecting rats by direct exposure. Irradiated cholesterol prevented the occurrence of rickets in rats on a low calcium rickets-producing diet as well as on the low phosphorus diet.

When human or calf skin was fed to rats daily in 1.0 gm. amounts it did not protect them against rickets, but when skin was fed which had been irradiated for a half hour the rats failed to develop this disorder.

Irradiated dry milk, patent flour and spinach were rendered antirachitic by means of irradiation with the mercury vapor lamp; the spinach was found to retain this quality after it had been subsequently boiled for one-half hour. Oleic acid and egg phosphatide failed to be activated by the rays.

Dihydro-cholesterol and dihydro-phytosterol, which are completely saturated and contain no double bonds, remained inert after irradiation. Nor did spectograms show any alteration in their absorption of ultra-violet rays as is the case with cholesterol and phytosterol. The unsaturated terpenes, cymene and citronellol, were not activated by irradiation.

¹ Hess, A. F., Weinstock, M., and Helman, F. D., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 227.

155 (2678)

The reaction of the protoplasm of the living amoeba to
injected salts.

By ROBERT CHAMBERS and PAUL REZNIKOFF.

[From the Department of Anatomy, Cornell University Medical
College, New York City.]

The salts, NaCl, KCl, CaCl₂, and MgCl₂, in various concentrations both separately and combined, were injected into the living *Amoeba* by means of the micromanipulation apparatus.¹ The bore of the micropipettes averaged between one and two micra in diameter, and the volume of fluid injected could be controlled to amounts varying from about half the volume of the nucleus to that of the entire *Amoeba*. *Amoeba proteus* is ideal for this micro-operative work, not only because of the ease with which it can be injected, but also because of its characteristic reactions to the fluids injected. All the salts introduced produce a momentary dilution, as indicated by a scattering of the cytoplasmic granules in the injected area.

When NaCl or KCl is introduced, the dilution is accompanied by a decided liquefaction of the injected region which becomes quiescent. The surrounding protoplasm then pours into this area where movement ceases. During this quiescent phase the larger and presumably heavier crystalline granules fall to the bottom of this region and there tend to clump against the lower surface of the *Amoeba*. Recovery takes place by a reappearance of currents in and around the quiescent area, and a gradually increasing flow, back and forth, until the *Amoeba* resumes its normal state. The size of the region involved is conditioned by the strength of the salt solution used, and by the amount injected. The *Amoeba* quickly recovers from an injection of a 2M solution of NaCl equal in amount to the volume of its nucleus. If more than this be injected the entire *Amoeba* is converted into a quiescent globule of dead, liquid protoplasm surrounded by a delicate pellicle which readily disrupts when torn with the micro-needle. The granular contents then scatter in the surrounding

¹ Chambers, Robert, *Anat. Record*, 1922, xxiv, 1.

medium. Injections of M/4 to M/8 NaCl produce only a momentary, localized quiescence, and the *Amœba* rapidly recovers, even from very large doses, by a rushing back and forth of the cytoplasm between the injected and uninjected regions. Solutions in concentrations weaker than M/8 produce effects in the *Amœba* which approach those occurring when water alone is injected.² KCl, except for being more toxic, closely resembles NaCl in its effect on the *Amœba*.

CaCl₂, on the other hand, produces an effect quite different from either that of NaCl or of KCl. The injection of CaCl₂ is immediately followed by a contraction and a solidification of the injected region. Strengths of CaCl₂ varying from 2M to M/2 when introduced in volumes from $\frac{1}{8}$ to $\frac{1}{2}$ of the *Amœba*, immediately set the entire *Amœba* into an irreversible solidified mass with protruded pseudopodia. Small amounts of 1M, M/2 and large amounts of M/4 to M/104 CaCl₂ produce only a localized solidification. Sometimes, especially with the stronger of these solutions, there is an immediate flow of protoplasm to the injected area with a consequent increase in volume of the involved region. The *Amœba* then subsequently reacts by a flow of its healthy protoplasm away from the involved region, which is thus left behind as an inert, solidified mass. By an active "pinching-off" process this mass is rejected by the *Amœba*. A surprising feature of the CaCl₂ injection is the rapid "pinching-off" reaction on the part of the *Amœba*. The area affected tends in this way to be eliminated, and leaves the rest of the *Amœba* apparently unaffected and normal. When MgCl₂ is injected, the protoplasm solidifies in much the same way as with CaCl₂. However, no "pinching-off" reaction takes place, and the solidifying process, instead of being limited, gradually spreads throughout the *Amœba*.

In brief, NaCl and KCl produce a liquefaction of the injected area, whereas CaCl₂ and MgCl₂ cause a solidification.³

Combinations of these salts in various proportions were also injected. It was found that NaCl and CaCl₂ in certain very definite proportions, *viz.*, 1M NaCl with M/52 CaCl₂, M/2 NaCl with M/104 CaCl₂, and M/4 NaCl with M/208 CaCl₂, antago-

² Chambers, Robert, Sect. V, General Cytology, Univ. of Chicago Press, 1924.

³ Chambers, Robert, 1924 Meeting of the Pathological Society, Federation of American Societies for Experimental Biology.

nize one another in such a manner as to neutralize the solidifying effect of the CaCl_2 and the liquefying action of NaCl . When combinations of KCl and CaCl_2 were injected it was found that these two salts neutralize one another when combined in the proportions of 1M KCl with M/26 CaCl_2 , M/2 KCl with M/104 CaCl_2 , and M/4 KCl with M/208 CaCl_2 .

It may, therefore, be inferred that at least one of the features of the antagonistic action of NaCl or KCl to CaCl_2 is the maintenance in protoplasm of a definite balance between its liquid and solid phases. This phenomenon possibly depends upon the formation of a balanced proportion of Na and Ca or of K and Ca protein salts. It may also be due to the formation of Na or K and Ca soaps.

156 (2679)

The lactic acid content of blood and spinal fluid.

By KIKUGORO NISHIMURA. (Introduced by J. A. Killian).

[From the Department of the Laboratories, New York Post-Graduate Medical School and Hospital, New York City.]

As a preliminary to the study of variations of the concentration of lactic acid in blood and spinal fluid in pathological conditions, it was found essential to establish the normal limits for human blood and spinal fluid. Clausen's method has been adopted as the most satisfactory procedure. This method comprises the following steps: The precipitation of the blood proteins by tungstic acid by the Folin-Wu procedure, and the removal of glucose from this filtrate by means of copper sulphate and calcium hydroxide; the extraction of the lactic acid from this glucose-free filtrate with ether; the oxidation of the lactic acid to acetaldehyde with potassium permanganate; the distillation of the acetaldehyde into sodium bisulfite solution, and the titration of the excess and combined bisulfite, with iodine. With this method, Clausen states the provisional figures for normal human blood are from 15 to 32 mg. per 100 cc. This indicates a variation of more than 100 percent. The object of the present

study was to investigate the lactic acid content of normal blood under standard conditions, eliminating all the known sources of error. The distillate is received into approximately 0.02 N NaHSO_3 . This solution keeps but a short time. It was prepared daily from 0.1N stock solution. Kahlbaum's sodium bisulfite has been used. The solution must be stoppered and preserved on ice. This 0.1N solution was daily standardized against iodine. For the determination of the excess bisulfite 0.02 N iodine was employed and for the bound bisulfite 0.01 N. The iodine must be daily standardized against $\text{Na}_2\text{S}_2\text{O}_3$, and this in turn is checked against $\text{K}_2\text{Cr}_2\text{O}_7$. It is necessary to use very pure NaHCO_3 . Kahlbaum's and Merck's reagent products were found satisfactory. The ether is probably the principal source of error. Merck's reagent ether with specific gravity of 0.720 gave the lowest and most constant blanks. With these reagents, the method gives blanks varying from 0.10 to 0.7 cc. of 0.01N iodine. Frequent blank and recovery determinations are necessary. For solutions of lactic acid in water, and lactic acid added to blood, in quantities to 120 mg. per 100 cc., the average recovery has been about 97 per cent. If the receivers are kept immersed in ice water, and the acetaldehyde is drawn over with gentle suction, about 75 per cent of the aldehyde is bound by the bisulfite in the first receiver, and the remainder in the second. A strong air current causes a loss of the aldehyde.

The length of time the blood has been standing after removal from the body before the determination is made, is an important factor influencing the results. Determinations are made on oxalated whole blood. If the glucose is removed from the filtrate immediately, no increase in lactic acid occurs during 24 hours at room temperature. If, however, the glucose is not removed, the lactic acid steadily rises. The average increase after 24 hours is about 50 per cent. There is noted also a slight decrease in the sugar. The whole blood on standing at room temperature shows a continuous increase in lactic acid with a drop in the sugar. After 3 hours, the increase may be 10 to 50 per cent; after 6 hours, 20 to 100 per cent, and after 24 hours, 75 to 400 per cent of the figure for fresh blood. The ingestion of carbohydrate produces a rise in the blood lactic acid, accompanying but not paralleling the increase in blood sugar. With the return to normal, the lactic acid lags behind the sugar. The

lactic acid of the blood has been determined in 30 cases, representing normals and hospital cases with no evident disturbance of carbohydrate, protein or fat metabolism. The bloods were obtained after a 14-hour fast, and after a night's rest, and analysed immediately. The figures varied from 11.7 to 18.0 mg., with an average of 15 mg. per 100 cc.

Clausen's method has been found applicable to the study of the lactic acid of spinal fluid. In human cases of encephalitis, brain tumor and cerebrospinal lues the lactic acid varied from 9 to 13 mg. per 100 cc. In tuberculous meningitis and meningococcus meningitis there was an increase exceeding 100 per cent of the normal, the figures were found to be as high as 22.5 mg.

157 (2680)

The influence of partial inactivation upon the potency of the bacteriophage.

By GREGORY SHWARTZMAN. (Introduced by I. S. Kleiner).

[From the Laboratory of Bacteriology, N. Y. H. Medical College and Flower Hospital, New York City.]

Bordet and Ciuca¹ believe that the bacteriophage phenomenon is a hereditary transmissible autolysis, in which the principle is generated by the bacteria themselves at a certain stage of their development under the influence of a similar lytic principle. Naturally, this conception requires the establishment of certain definite relationships between the amount of lytic principle which acts upon bacteria and the energy of the principle generated by bacteria. In their studies of these relationships Bordet and Ciuca made the following observations: The addition of a rather small amount of anti-colon bacteriophage to *B. coli* will result in only slight lysis. Furthermore, this minimal amount of the principle²

¹ Bordet and Ciuca, *Compt. Rend. Soc. de biol.*, 1922, lxxxvi, 295, and 1922, lxxxvi, 366 and 987.

² The lytic principle has to be diluted 10^{-8} according to Gratia, *Compt. Rend. Soc. de biol.*, 1923, lxxxviii, 629.

will induce the bacteria to generate a new principle of a weak potency. No matter what amount of this weak principle is now used, only a weak lysis will occur and a weak principle will be generated. To explain these observations these authors assume that the introduction of a very small amount of lytic principle (quantitative reduction) leads to its considerable dispersion among the bacterial cells. Therefore each bacterial cell is only very weakly impressed by this dispersed principle and thus reacts with a generation of a new principle of low energy (qualitative reduction).

It occurred to the author of this paper that instead of dispersing the lytic principle among the bacterial cells, it would be of interest to study the relationship of a partially inactivated lytic principle to the energy of generation of this principle by bacterial cells.

A method had to be chosen for partial inactivation of the lytic principle.

Since it is known that a certain hydrogen ion concentration (pH 2.8) inactivates completely the lytic principle, it was thought that a certain range of pH close to the inactivating zone could be found in which the lytic principle would be only partially inactivated.

With this intention the influence of buffer mixtures in the pH range 2.8 to 4.8 on activity of bacteriophage was studied. The following table represents the results obtained.

As is seen, partial inactivation of the lytic principle can be obtained by this method.

It is, however, impossible to state whether this partial inactivation is of a qualitative or quantitative nature. According to the experiments of Bordet and Ciuca the partially inactivated principle, be it quantitative or qualitative, should ultimately generate only a weak lytic principle. This, however, was not found to be the case, as is seen in the following experiment:

Tube of broth culture of *B. coli*, in which lytic principle previously exposed to the action of buffer pH 4.2, was diluted 10^{-4} and which showed only two plus of lysis (cf. Table), was sterilized by heating to 58° for one-half hour and the fluid distributed into a series of tubes of broth in dilution from 10^{-1} up to 10^{-8} . All these tubes were then inoculated with *B. coli*. In 24 hours *complete lysis* was obtained in all the tubes of this series.

TABLE.

pH of buffers to which anti-colon phage is exposed in dil. 1:100 room temp. 48 hours.	Degrees of lysis in tubes of broth in which each buffer mixed with phage was diluted after adjustment to pH 7.0 and into which <i>B. coli</i> was inoculated.					
	Dil. 10 ⁻³	Dil. 10 ⁻⁴	Dil. 10 ⁻⁵	Dil. 10 ⁻⁶	Dil. 10 ⁻⁷	Dil. 10 ⁻⁸
2.8-3.6	—	—	—	—	—	—
3.8	1+	—	—	—	—	—
4.0	2+	1+	1+	1+	1+	—
4.2	3+	2+	1+	1+	1+	—
4.4	3+	2+	2+	1+	—	—
4.6	4+	3+	2+	2+	1+	—
4.8	4+	3+	3+	2+	2+	1+

Reading in 24 hours.

4+ = Complete lysis.

3+ = Moderate lysis.

2 and 1+ = Slight lysis.

— = No lysis.

To sum up: I. Partial inactivation of the potency of lytic principle can be obtained by exposing the principle to the action of buffer mixtures of certain hydrogen ion concentrations at room temperature for 48 hours.

II. The lytic principle, partially inactivated by this method, induces, in contrast to the results obtained by Bordet and Ciuca, the generation of a principle of *maximal activity*.

158 (2681)

The action of some derivatives of ergot in peripheral vaso-motor exhaustion.

By HELEN C. COOMBS.

[*From the Department of Physiology, New York University and Bellevue Medical College, New York City.*]

In a previous report on the effects of adrenalectomy on the number and duration of anemic responses to temporary occlusion of the head arteries in the cat,¹ it was shown that the failure of blood-pressure to rise above spinal level was due to failure at the myo-neural junction, and not to exhaustion of the medulla; in animals subjected to adrenalectomy, injection of small doses of adrenalin so restored general conditions that the cardio-vascular and nervous mechanisms again became functionally competent, and several more occlusions could be done before there was again failure at the periphery. If, after subsequent failure of vaso-motor response, adrenalin was again injected, several more rises of blood-pressure could be obtained on occlusion of the head arteries.

The question arose as to whether the specific action of adrenalin was necessary to restore vaso-motor tone under such conditions, or whether any vaso-constrictor, acting at the myo-neural junction, would be effective. In several animals, therefore, after adrenalectomy and the typical peripheral failure of blood-pressure, tyramine (Burroughs-Wellcome), a preparation of ergot, was injected intravenously, under conditions similar to those in which adrenalin had been injected. In every case, there was an immediate and very adequate vaso-constrictor effect. Blood-pressure rose, and although it did not attain the height produced by the injection of adrenalin, it was maintained at a high level for a much longer period of time and was equally effective. The corneal reflex returned, and spontaneous respiration gave evidence of restored bulbar function. The anemic rise in blood-pressure could then be elicited several times before pressure again fell permanently. With another injection of tyramine, the entire process could be repeated, and so on until bulbar exhaustion was produced.

¹ Coombs, H. C., *Am. J. Physiol.*, March, 1925. (In press.)

Fluid extract of ergot (John Wyeth and Bro.) was likewise injected in doses of 15-30 minims in 5 cc. of Ringer's solution, under similar conditions of peripheral failure; but although there was a slight rise in blood-pressure, it was in no way comparable to that produced by tyramine, either in height or duration, and completely failed to effect any restoration of cardiovascular function.

159 (2682)

The rôle of the dorsal spinal nerve roots in bulbar anemia.

By HELEN C. COOMBS.

[*From the Department of Physiology, New York University and Bellevue Medical College, New York City.*]

Pike¹ has shown that in the spinal cat there is a certain reflex element concerned in the maintenance of blood-pressure, by the fact that section of the dorsal roots of the spinal nerves in the thoracic region causes a fall of about 10 millimeters of blood-pressure. Wickwire² has demonstrated that section of the dorsal roots of the spinal nerves from C5 to L2 does not abolish the compensation of heart rate to high and low blood-pressures.

A series of experiments was made to see whether division of the dorsal roots of the spinal nerves would affect the cardiovascular response to bulbar anemia, produced by temporary occlusion of the arteries to the head.³ Laminectomy was done through the thoracic region, and the head arteries were isolated as usual. The blood-pressure rise in a control occlusion of the head arteries was then recorded, and as soon as recovery was initiated, the dorsal roots were divided in the thoracic region, and occlusion of the head arteries was repeated. Such difference in response as was shown between the two occlusions was due to the low pressure which resulted from the extensive operation,

¹ Pike, *Quart. J. Exper. Physiol.*, 1913, vii, 1.

² Wickwire, *Am. J. Physiol.*, 1920, liii, 355.

³ Stewart, Guthrie, Burns and Pike, *J. Exper. M.*, 1906, viii, 289.

and not to any intrinsic difference in the mechanism of the response. Few responses could be elicited owing to the persistent low level of the blood-pressure, although respiratory gasps indicated that the medulla was not exhausted. Injection of adrenalin or tyramine caused a sufficient vaso-constriction to raise blood-pressure enough to restore the medulla, so that further anemic responses could be elicited; but in general, the number and magnitude of these responses was far below normal. This difference was probably due not to any interruption of an essential part of the vaso-motor pathway, but to the general condition of the organism.

160 (2683)

The distribution of the immune bodies occurring in Types I, II and III antipneumococcus serum.

By EDWIN J. BANZHAF.

[From the Bureau of Laboratories, Health Department, New York City.]

Avery¹ states that the immune bodies occurring in Types I and II are completely precipitated by 38 to 42 percent saturation with ammonium sulphate, and that they were incompletely precipitated by (a) ammonium sulphate in less than 38 percent saturation, (b) saturation with sodium chloride, (c) dilution and saturation with carbon dioxide and (d) removal of crystalloids by dialysis. He states that the most practical purification appears to be precipitation by 38 to 42 percent saturation with ammonium sulphate. The higher saturation, *i. e.*, the 42 percent, corresponds to about 47.6 cc. of saturated ammonium sulphate solution.

Felton² finds that a 10 times dilution with distilled water containing 4 percent N/1 phosphoric acid per volume of antiserum, will, with Type I, completely precipitate the immune bodies. He has been less successful with some of the antisera of Types II and

¹ Avery, O. T., *J. Exper. M.*, 1915, xxi, 133.

² Felton, L. D., *Boston M. & S. J.*, 1924, cxc, 819.

III, especially those of low protective value. We have corroborated Felton's findings with Type I. Practically a complete precipitation of the immune bodies occurs when diluted with distilled water containing 4 percent N/1 phosphoric acid per volume of antiserum. Felton's same technique on Types II and III has given us varying results.

Using ammonium sulphate as a precipitating agent, we corroborated Avery's work in finding that the immune bodies were completely precipitated by half saturation with saturated ammonium sulphate solution. We also found that the methods of separation of the so-called euglobulin and pseudoglobulin by ammonium sulphate and sodium chloride showed immune bodies in both globulins. We found, however, that that portion of so-called euglobulin which is precipitated with 30 percent saturated ammonium sulphate solution, saturated sodium chloride or 12½ percent dried sodium sulphate, contained after dialysis only 8 to 10 percent of the total immune bodies. This holds true for all three types.

Using Felton's technique for diluting these dialyzates, the white precipitate that is obtained contains practically all the immune bodies from these first fraction precipitates or so-called euglobulins.

The filtrate containing the globulins soluble in 30 percent saturated ammonium sulphate solution, and precipitated by adding saturated ammonium sulphate solution to half saturation, contains after dialysis about 90 percent of the immune bodies.

The saturated sodium chloride soluble globulins, precipitated by the addition of only 25 percent saturated ammonium sulphate solution, contains after dialysis about 90 percent of the immune bodies. The filtrate containing the globulins soluble in 12½ percent dried sodium sulphate, and precipitated by adding dried sodium sulphate up to 18½ percent at 36° C., also contains, after dialysis, about 90 percent of the immune bodies.

Using Felton's technique for diluting these dialyzates, the white precipitate that is obtained contains practically all the immune bodies from these second fraction precipitates. This holds true for all three types. In all instances where Felton's white precipitate is obtained in the three types of pneumococcus antisera, the immune bodies are found in his white precipitate.

The albumins have no immune bodies, and no white precipi-

tate appears when diluted with distilled water. Heating the antisera to 58° C. for two hours destroys about 60 percent of the immune bodies in all three types.

Heating to 56° C. for two hours does not impair its protection value.

All protection tests were made by Miss W. Carey Noble, to whom I am greatly indebted.

161 (2684)

On the function of the colonic spindle (*Fusus coli*) of the rabbit.

By JOHN AUER.

[From the Department of Pharmacology of the St. Louis University School of Medicine, St. Louis, Mo.]

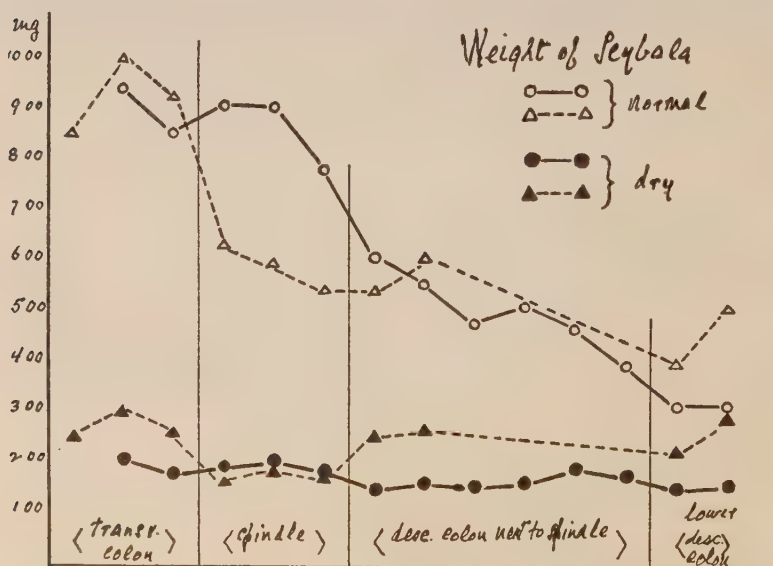
In previous notes¹ attention was drawn to an apparently undescribed, macroscopic, spindle-shaped, sphincter-bearing structure which connects the transverse colon of the rabbit with the descending colon.

One of the main functions of this organ apparently is to prevent, under normal conditions, the passage of scybala before they have been deprived of most of their water content. This seems to be accomplished largely by mechanical pressure exerted on the moisture-soaked scybala by the muscular spindle, the passage of the pellet into the descending colon being prevented by contraction of the sphincter at the spindle neck.

Evidence for this action is furnished by inspection of the active spindle in the living animal, and by inspecting and weighing the scybala in the order of their location in the transverse colon, the spindle and in the descending colon. In the living rabbit under morphin narcosis, where peristalsis has been accelerated by the intravenous injection of 0.1 to 0.3 mg. of physostigmin, one may occasionally see a spurt of fluid spiralling through the neck of the spindle into the descending colon, as a powerful per-

¹ *J. Pharmacol. and Exper. Therap.*, 1925, *Proc. Soc. Pharmacol. and Exp. Therap.*, and *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 301.

istaltic contraction of the spindle tries to drive the mass through the closing sphincter. Obvious differences in the size, appearance and consistency of the various scybala in the different sections of the region in question also indicate the same fact. The most conclusive evidence of this mechanical dehydrating action of the spindle is however obtained when the scybala of these regions are weighed. The accompanying graph illustrates this statement; the normal weights are those which were obtained immediately after autopsy; the dry weights of the same scybala are those obtained after drying to a practically constant weight at room temperature. The weights on the same ordinate with the same symbol represent the same scybalum in the normal and the dehydrated state; those on different ordinates are different scybala; only two series of observations from different animals are recorded, as they well illustrate what is generally found under normal conditions. It will be noticed that the weight of the normal scybala drops abruptly in the spindle, and this loss of weight continues at a slower rate in the descending colon immediately adjoining the spindle. That this loss of weight is due to the loss of water is indicated by the relatively equal weight of the



Weights of the various scybala in their order of occurrence in the different sections of the colon of the rabbit. The weights are recorded as found on immediate autopsy and after drying to a constant weight.

various scybala when dried (see graph). This relative equality of dry weight of scybala in the different sections of the gut may be most striking in some animals (see circle-bar series in graph); in other animals the variation in weight of the dry fecal residue is more pronounced.

The differences in the normal weight of scybala briefly described above are not found in those rabbits where peristalsis of the colon has been hurried by drugs or by disease processes.

Whether or not the rate of absorption varies in the different colonic segments will be discussed at another time.

The diet of the rabbits consisted chiefly of oats and hay, with some green stuff; water was available at all times.

162 (2685)

The relation of contractile and food vacuoles to rhythms in *Paramecium*.

By W. BYERS UNGER. (Introduced by L. L. Woodruff).

[From the Osborn Zoological Laboratory, Yale University, New Haven, Conn.]

Experiments were undertaken to determine the relation between metabolic activity, as indicated by the rate of contractile vacuole pulsations and the size and number of food vacuoles formed, and the occurrence of rhythms in the division rate. The animals studied were pedigree races of *Paramecium aurelia* and *Paramecium calkinsi*. Two cultures of each species were studied for 115 days under constant culture conditions. Observations were made daily and averages computed for the per diem division rate, food vacuole number and size, and the pulsation rate of the anterior and posterior contractile vacuoles.

In *Paramecium aurelia*,¹ depressions in the rate of contractile vacuole pulsation are, in general, synchronous with the low points of the rhythms in the division rate and with the occurrence of endomixis. The posterior contractile vacuole pulsates

¹ Woodruff, L. L., *Biol. Bull.*, 1917, xxxiii, 51.

more rapidly than the anterior. The variations in the number of food vacuoles formed are, in general, synchronous with the rhythms in the division rate and the fluctuations in the rate of pulsation of the contractile vacuoles.

Although endomixis does not occur in *Paramecium calkinsi*,² this species exhibits definite fluctuations in the rates of the two contractile vacuoles which corresponds to the rhythms in division rate. The anterior and posterior contractile vacuoles show no significant difference in rate. As in *P. aurelia*, the fluctuations in the number of food vacuoles formed in *P. calkinsi* is synchronous with the rhythms in reproductive activity and the fluctuations in contractile vacuole pulsations.

Paramecium calkinsi forms more food vacuoles than *Paramecium aurelia*, though the size of the vacuoles is smaller in the former. The rate of pulsation of the contractile vacuoles is faster in *P. aurelia* than in *P. calkinsi*; a difference probably correlated with the smaller size of *P. calkinsi*.

The fluctuations in the several phenomena observed are not caused by endomixis since rhythms are present in *P. calkinsi* (where endomixis does not occur) as well as in *P. aurelia* (where endomixis does occur). Apparently the rhythms in *P. aurelia* are accentuated by endomixis since the changes in the rate of the contractile vacuoles, in particular, are more abrupt and extend over a greater range than those of *P. calkinsi*.

In brief, fluctuations in nutrition and excretion, as indicated by food vacuole formation and contractile vacuole pulsation, are shown to be coincident with, and probably causally related to, the well-established rhythms in the reproductive activity of *Paramecium*.

² Spencer, H., *J. Morph. and Physiol.*, 1924, xxxix, 548.

163 (2686)

A preliminary note on the physiology of the uterine opening of the Fallopian tube.

By FERDINAND C. LEE.

[From the Anatomical Laboratory of the Johns Hopkins University, Baltimore, Md.]

In an attempt to obtain evidence regarding Sampson's¹ theory for the etiology of adenomas of endometrial type found in the lower abdominal cavity, the cervical portion of the uterus in several bitches was occluded under aseptic precautions. It was hoped that the periodic sanguinous uterine discharge would thus be forced to find its way through the Fallopian tubes into the abdominal cavity. Then, if adenomas lined by Müllerian epithelium were found, it would be reasonable to believe that the uterine discharge contributed to their formation. However, successive laparotomies after the oestral period showed that the material did not leave the uterus, but simply distended that organ. Obviously the question arose as to the nature of that mechanism which allowed spermatozoa to pass at one time but prevented secretions from passing at another. Accordingly, injections with India ink were directed into the cornu of the uterus towards the Fallopian tube in the cat. It was found that when the uterus was small and the ovaries showed no large follicles, ink would pass into the tube but under great difficulty, a pressure of 280 mm. of mercury being frequently necessary. On the other hand, in the same animal, injections into the isthmus of the tube and directed towards the cornu passed easily into the uterus. However, when the uterus was large and swollen, and when relatively large follicles appeared in the ovary, then the passage from uterus to tube was easy. Experiments along the same lines on the dog and guinea pig have so far indicated the same general phenomenon.

The recent report of Rubin² on the various pressures necessary for transuterine insufflation of the tubes at various stages in the

¹ Sampson, J. A., *Surg. Gynec. and Obst.*, 1924, xxxviii, 287.

² Rubin, I. C., *J. Am. Med. Assn.*, 1925, lxxxiv, 486.

intermenstrual period of the same individual, is in harmony with the general principle obtained from work on the lower mammals. It is believed that the uterine end of the tube, through its varying degrees of patency, is to a great extent responsible for the differences in pressure he obtained. Furthermore, the material examined thus far indicates that the greatest patency occurs about the period of ovulation, probably a little before that time.

164 (2687)

The locus of the action of veratrin in the sciatic nerve of the frog.

By CHARLES L. WIBLE. (Introduced by A. R. Moore).

[*From the Physiological Laboratory of Rutgers University,
New Brunswick, N. J.*]

In a former paper¹ it has been pointed out that if the end of a sciatic nerve is immersed in a veratrin solution there results, after a widely variable latent period, spontaneous muscle twitchings in the gastrocnemius. If, at this time, the sciatic nerve is sectioned between the point of immersion in the veratrin solution and attachment to the muscle, the writing lever falls to the base line. It has also been shown that direct stimulation of the gastrocnemius, when the nerve is immersed, elicits a contraction with delayed relaxation. The question arises as to whether some of the veratrin solution actually travels along the nerve fiber to the muscle by capillarity and thus functionally affects the muscle fibers. If this should be the case, the wide range of latency might be accounted for by rapidity of transmission or length of nerve. Moreover, with this conception of the phenomenon, a nerve-block applied between the supply of veratrin and the muscle should not inhibit muscular twitchings due to chemical stimulation by veratrin, nor characteristic contracture of the muscle, following direct electrical stimulation. In order to test this theory experiments were made with nerve muscle preparations of the frog arranged as previously described¹ and using the same con-

¹ Wible, C. L., *J. Gen. Physiol.*, 1924, vi, 615.

centration of alkaloid solution. An ethyl chloride nerve-block (Gebauer's ethyl chloride) was employed between the veratrin container and the muscle.

If the nerve-block be applied before the nerve is immersed in veratrin solution, direct stimulation of the muscle over a period of 45 minutes results in normal contractions. If, after the nerve has been immersed in veratrin and the muscle twitchings have begun, the nerve-block is now applied, the writing lever returns at once to the base line. Direct stimulation of the gastrocnemius at this time and at intervals over a period of one hour elicits only normal contractions. In the above experiments the nerve-block was known to be complete, since mechanical stimulation of the nerve did not result in contraction of the muscle.

The results obtained show that the characteristic reaction of the gastrocnemius, as a result of the immersion of the sciatic nerve in a veratrin solution, is inhibited by an ethyl chloride nerve-block. For this reason it must be concluded that there is no actual transmission by capillarity of the alkaloid to the muscle by the nerve fiber. And therefore rapidity of transmission or length of nerve does not play a part in the wide range of latent period. This evidence also confirms the interpretation of former observations, namely, that the drug has an excitatory action only on that portion of the medullated nerve fiber which is actually immersed in solution.

165 (2688)

The effect of surface tension on the growth of *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*.

By W. R. ALBUS and GEORGE E. HOLM.

[From the Research Laboratories of the Bureau of Dairying, U. S. Department of Agriculture, Washington, D. C.]

The close similarity, both morphologically and culturally, between *Lactobacillus bulgaricus* and *Lactobacillus acidophilus*, makes differentiation between these two important members of the aciduric group of bacteria difficult. Their similarity is so

close as to have led some investigators^{1, 2} to conclude from earlier work that they were identical. A differentiation based on the fermentation of maltose,³ and maltose, saccharose and levulose,⁴ is admittedly more or less variable. Unpublished work done in these laboratories on sugar fermentations of these organisms likewise gave variable results.

It would seem, then, that a differentiation of these two types of organisms based on sugar fermentations is not absolutely infallible, and a method of distinguishing between these two closely related organisms is most desirable.

A review of the voluminous literature on the implantation of aciduric bacteria in the intestinal tract seems to justify the conclusion that *Lactobacillus bulgaricus* cannot be implanted in the intestinal tract, while implantation of *Lactobacillus acidophilus* is more or less easily accomplished. That the bile salts may play some part in this natural selection suggested itself, and as they are surface depressants, separation on this basis was attempted. Fifteen strains of *Lactobacillus bulgaricus* and fifteen strains of *Lactobacillus acidophilus* gathered from authentic sources were employed in this work. Sodium ricinolate and sodium taurocholate were used as surface tension depressants in a medium favorable for growth of all strains. All of our strains of *Lactobacillus acidophilus* grew very well in a medium of a surface tension as low as 36 dynes, while *Lactobacillus bulgaricus* in the same medium depressed to a surface tension of forty dynes, failed to show growth after seven days' incubation at 37° C. A surface tension of forty dynes represents the extreme lower limit for *Lactobacillus bulgaricus* as most of our strains were inhibited above this value.

This, then, offers a means of differentiating *Lactobacillus bulgaricus* from *Lactobacillus acidophilus*, which separated, without exception, all of the strains employed in this work.

That surface tension may be a factor in the implantation of these organisms seems plausible.

¹ Rodella, *Centralbl. f. Bacteriol. Abt. I. O.*, 1901, xxix, 717.

² Heinemann and Hefferen, *J. Inf. Dis.*, 1909, vi, 304.

³ Rahe, *J. Inf. Dis.*, 1914, xv, 141.

⁴ Kulp and Rettger, *J. Bact.*, 1924, ix, 357.

PACIFIC COAST BRANCH

*University of California Hospital, San Francisco, Cal.,
February 18, 1925*

166 (2689)

The regression of age with size, a neglected aspect of growth.*

By F. W. WEYMOUTH, H. C. McMILLIN, and WILLIS H. RICH.

*[From the Laboratory of Physiology, Stanford University, Cal.,
and the United States Bureau of Fisheries, Washington, D. C.]*

Growth, always an attractive study, has of recent years received the attention of several distinct types of workers who have amassed much data for man, for some domesticated animals of economic importance, for the ever-useful white rat and guinea pig, and for a very few invertebrates. Almost invariably the results have been presented as average weights or lengths at stated ages. Thus presented, the growth data of most animals agree in certain general features. There is an early period of rapid growth which gradually slackens with age. In some forms growth continues, though at a very reduced rate, throughout life; in others, notably in man and birds, growth completely ceases for a long period of adult life. This is followed by a period of declining size.

A graph constructed from these values shows the average length (or weight) for any age. Recently while working on the growth of the razor clam, a bivalve of considerable commercial value, which is in need of protective legislation, a growth curve of this kind was used in studying the possible effects of different proposed legal sizes, and, as often happens in work of this sort, it became desirable to determine the average age of clams of different lengths. At first it would appear that the same graph contained these values if the process of reading were merely reversed. Is such a process correct?

The correlation between age and weight or length is non-linear; the correlation ratio, as usually calculated, is very high, often exceeding 0.9. As is well known in ordinary linear correlations, the line of regression of x on y is not the same as that of

* Published by permission of the Commissioner of Fisheries.

y on x , though with correlations above 0.9 the difference is not great. A moment's reflection, however, will show that the present case is less simple, and that the correlation is not the same at all ages. In early life, size increases rapidly with age, and a high correlation might be expected; when, however, an age is reached beyond which length or weight does not increase, the correlation must fall.

In order to see what relation actually obtains in different parts of the course of growth, we have calculated for the razor clam, in addition to the customary average length for each age, the average age for each length, and both of these regressions are presented in Figure 1. As we anticipated, the two curves were very nearly coincident in the early part; but as the curve of average length flattens out, the curve of average age rises above it, flaring up sharply toward the end. As a result while the average length of four-year-old clams is 9.2 cm., and the average age of clams 9.2 cm. long is four years, the average length of 11.0 year old clams is 15.25 cm., but the average age of clams 15.25 cm. long is only 9.5 years; thus an error of 1.5 years would result from reading the age from the curve of average length in this part of its course.

Since the effect of the reduction of growth with age is far more marked in man, the discrepancy between the two curves might be expected to be greater here. We were unable to find

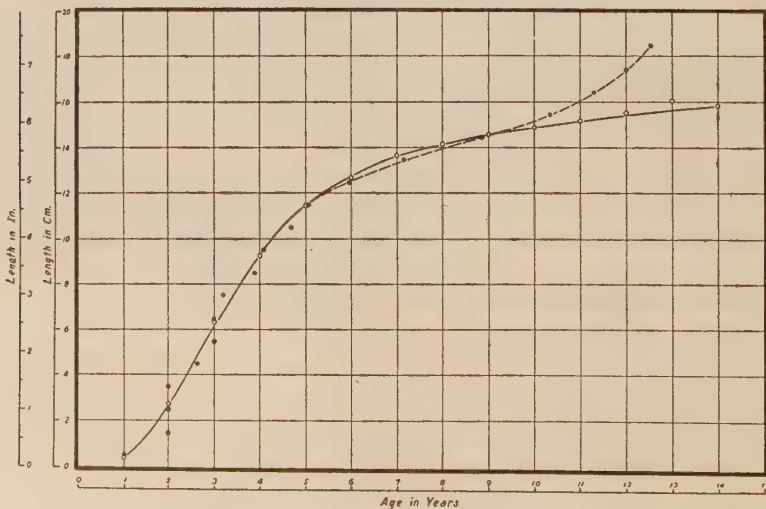


FIG. 1. The Growth of the Razor Clam.

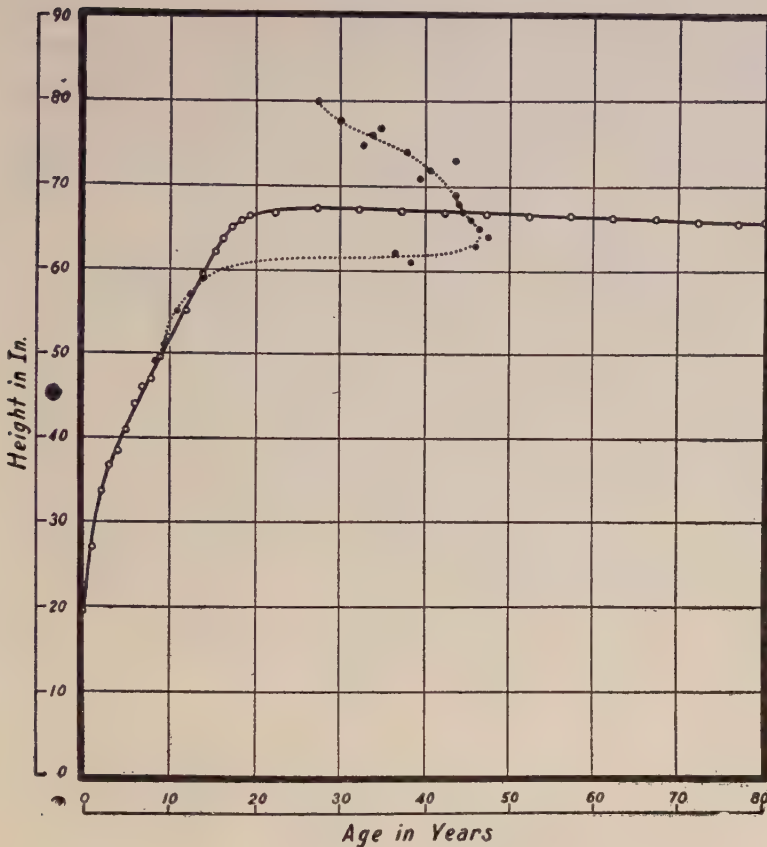


FIG. 2. The Growth of Man (English Males).

records of average ages for different heights or weights, and it proved surprisingly difficult to locate data from which they could be calculated. When located, the data required careful treatment since the mortality during adult life greatly reduces the number of individuals contained in the older age classes, but has a lesser effect in reducing the abundance of the larger size classes. It was finally decided to give equal weight to all of the age groups by reducing the number of individuals for the included heights to percentages. Though some of the details are unsatisfactory, owing to the necessity of combining two sets of data,^{1, 2} the gen-

¹ Powys, A. O., 1902, Data for the problem of evolution in man, *Biometrika*, Vol. 1, Oct., 1901, Aug., 1902, pp. 45-47.

² Baldwin, Bird T., 1921, The physical growth of children from birth to maturity, *Studies in Child Welfare*, Vol. 1, No. 1, pp. 149, and (Roberts) 267.

eral features are clear and very striking. Of course no new data are involved, but some of the relations appear in an unusual aspect, and it seems probable that this method of presentation will aid in the analysis of growth.

We have first a period of rapid growth with a high positive correlation between age and height. When a height is reached which first includes a considerable number of adults—approximately the minimum adult stature—there occurs a sudden transition to a condition in which each increasing height gives a younger average age and each age a lower average height, that is, a condition of fairly high negative correlation. It is thus clear that a correlation ratio between age and height calculated for the entire span of man's life has little meaning. There are two distinct periods: One of youth up to the point where growth in height ceases, characterized by a high positive correlation, and a second period from the attainment of adult stature to death, characterized by a moderate negative correlation. This negative correlation in adult life apparently results from two factors. One is the actual decrease in height (and weight) of the individual with increasing age. The other is the effect of selective mortality. Persons of extreme height (there are authentic records of heights up to 282 cm.) or weight (which may exceed 600 lbs.) represent for the most part pathological cases and are not long lived; hence the low average age of the tallest men. This is borne out by the refusal of the insurance companies to accept as risks men above a certain size.

In conclusion we wish to emphasize two points: One is the danger of reading from growth curves other values than those specifically contained. The second is the incomplete picture of growth presented by the ordinary graph showing the average height or weight at each age, and the opportunity for further analysis offered by the simultaneous study of the converse relation, the average age for each height.

167 (2690)

Platelet deficiency a factor in diminished coagulability of the blood in anaphylaxis.

By E. W. SCHULTZ.

[*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.*]

Delay or loss of coagulability of the blood in anaphylaxis has long been recognized. It is generally attributed to excess of antithrombin or to diminished thromboplastin. While making a study of the blood fibrin in canine anaphylaxis by the method of Foster and Whipple¹ observations were made which we believe throw further light on the phenomenon. In making the blood fibrin studies, approximately 9 cc. of blood, obtained by cardiac puncture, are discharged into a centrifuge tube containing 1 cc. of a 1.5 per cent sodium oxalate solution. The samples are then centrifuged at moderate speed for thirty minutes. Exactly 2 cc. of the oxalated plasma is discharged into 40 cc. of clotting solution. This consists of 0.125 per cent calcium chloride in physiologic salt solution. Normal oxalated plasma usually forms a jelly-like coagulum within one hour, whereas the plasma in fatal anaphylaxis may remain uncoagulated for days. Occasionally a feeble coagulum forms after several days. During the course of the work several thromboplastic agents were employed, but it was found that a small quantity of platelets obtained from normal plasma gave us the most trustworthy results. They are obtained as follows: Several hundred cubic centimeters of normal oxalated blood obtained by cardiac puncture from a fasting dog is centrifuged at a slow speed until the red cells have been well sedimented and a more or less turbid plasma remains. The plasma is then pipetted off into 15 cc. centrifuge tubes, and centrifuged at high speed (2700 r.p.m.) for about one hour. At the end of this time several millimeters of a pearly white sediment will be found in the tips of the tubes. This sediment on microscopic examination proves to be composed chiefly of platelets. The tubes are carefully rinsed to remove adherent plasma, and the sediment is dispersed in a little saline by vigorous shaking.

¹ Foster, D. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, Ivii, 365.

An opalescent fluid results, which on being added to the clotting solution containing the anaphylactic plasma usually brings on coagulation within a few minutes, while the controls often fail to show coagulation for days.

It has also been possible to induce coagulation with the sediment obtained from the anaphylactic plasma of the same animal, but here it is necessary to pool the sediment from several tubes to get anything like the prompt coagulation which one may obtain from a single tube of normal plasma. The sediment obtained from anaphylactic plasma is appreciably less than that obtained from normal plasma. This would seem to confirm the opinion that the diminished coagulability is due primarily to a reduction in the number of platelets, which normally supply the necessary thromboplastin. These observations are in accord with those of Achard and Aynaud² and Pesci,³ who noted a reduction in the number of platelets in anaphylaxis. The reduction is considered to be due to clumping of the platelets as a result of physico-chemical disturbances. This explanation is supported by the observations of v. Behring,⁴ who found clumped platelets in the capillaries and smaller arterioles of the pia and the choroid plexus in guinea pig anaphylaxis. Studies on the actual platelet count in canine anaphylaxis and on the fate of the platelets will be undertaken shortly.

² Achard, Ch., and Aynaud, M., *Compt. rend. Soc. de biol.*, 1909, lxvii, 83.

³ Pesci, E., *J. de physiol. et path. gen.*, 1921, xix, 242.

⁴ v. Behring, E., *Deutsch. med. Wchnschr.*, 1914, xl, 233.

168 (2691)

Immunological studies on certain albuminoids.

By CARL L. A. SCHMIDT and JAMES FRUG.

[*From the Division of Biochemistry and Pharmacology, University of California, Berkeley, Cal.*]

Under the term "albuminoids" a group of substances are classified whose chief properties appear to consist in insolubility in all neutral solvents and resistance to enzymatic digestion. Solubility can be effected only through the agency of strong acids and alkalis. No work on the ability of these substances to act as antigens appears to have been carried out. Since solubility is an essential factor in the production of immune bodies as well as in the demonstration of their presence it might, *a priori*, be assumed that the members of this group are non-antigenic. The experiments herein described were carried out for the purpose of obtaining experimental data relative to the ability of several members of this group to act as antigens.

The fibroin was obtained from silk cocoons. The water-soluble proteins were extracted by repeatedly washing the silk in cold water, then autoclaving, and repeatedly washing with fresh portions of boiling water. The spongin was similarly treated. In order to induce production of immune bodies, 50 to 100 mgs. of fibroin were placed in the peritoneal cavity of each of three rabbits, and four rabbits were similarly treated with spongin. Attempts to demonstrate the presence of fixation antibodies at the end of a month were wholly negative. In carrying out the test a small arbitrary amount of "antigen" was employed. After addition of serum and alexin the mixture was incubated for several hours to give the maximum opportunity for the fixation of alexin, and allowances for the mechanical absorption of the added constituents by the spongin were made by the aid of a large number of control determinations with normal sera. Attempts to demonstrate immune bodies by skin tests were likewise negative. The autopsy findings showed masses of unaltered fibroin or spongin encapsulated by vascular connective tissue.

Another series of experiments was carried out with friable fibroin which was prepared in accordance with the suggestions of

Städeler¹ by treating fibroin with 5 per cent NaOH and subsequently neutralizing with HCl. The material so obtained can be finely ground, and by using a large needle a suspension of this substance can readily be injected intraperitoneally. Two rabbits received 7 doses of 100 mg. each over a period of two weeks, and two others were given 8 injections of 25 mg. each over a period of two months. Attempts to demonstrate the presence in the blood stream of fixation antibodies were entirely negative.

169 (2692)

Studies in tuberculosis. V. Phosphatids of tuberculin with diagnostic and sensitizing properties.

By FREDERICK EBERSON.

[*From the Department of Medicine, University of California, San Francisco, Cal.*]

In a series of earlier publications¹ an alcohol-soluble and ether-precipitable substance, "Ether-insoluble X," was described in connection with experiments on sensitization and diagnosis in animals and in tuberculous patients. Preliminary qualitative procedures pointed toward identifying this derivative with a phosphatid, probably lecithin, which represented a small fraction of the original old tuberculin. Typical tuberculin reactions were elicited, notwithstanding the exceedingly high dilutions that were used in the tests. It was suggested that the antigenic radical of *B. tuberculosis* might reside in such a fraction, and that the isolation of this derivative from tuberculin might offer a lead in the direction of explaining the mechanism of tuberculin reactions and the phenomena of sensitization which I have reported previously.

The present report is a chemical study of some lecithin-like fractions which have been obtained from old tuberculin (human)

¹ Städeler, G., *Ann. d. Chem. and Pharm.*, 1859, iii, 12.

¹ Ebersson, F., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxi, 539; *Am. J. Dis. Child.*, 1925, xxix, 29; *Am. Rev. Tuberc.*, 1925, March (in press).

prepared from beef-extract media,* and from two different synthetic substrates which were described by Löwenstein and Pick,² and by Gessard and Vaudremer.³ Quantitative analyses of the derivatives from these protein-free media will be published shortly. Qualitative studies have indicated that all of these derivatives are identical, and give typical skin tests regardless of the kind of media used in the preparation of tuberculin.

Tuberculin, in weighed 50 gram portions (49 cubic centimeters), was added drop by drop from a burette to pure methyl alcohol until complete precipitation was effected. Nineteen volumes of alcohol were required for each volume of tuberculin. To the clear filtrate which yielded no further precipitate, was added pure ethyl ether until complete precipitation occurred. This procedure required 3.4 volumes of ether for each volume of alcohol filtrate. The yield by alcohol precipitation from 50 grams of tuberculin was 7.45 grams dry weight, representing 14.9 per cent of the original tuberculin. Ether precipitation from 980 cubic centimeters of the alcoholic filtrate yielded 6.88 grams of substance, or 13.8 per cent of the original tuberculin. The ether residue obtained by evaporation and drying yielded 1.62 grams from 3,000 cubic centimeters of ether filtrate.

The alcohol precipitate is chocolate-brown in color, is freely soluble in water and in physiologic salt solution, from which it can be re-precipitated upon addition of alcohol. It contains 11.3 per cent Kjeldahl Nitrogen.

The ether-insoluble fraction obtained from the alcoholic filtrate is pink in color, browns on exposure to air, and the precipitate is gummy and hygroscopic. Repeated washing of the substance with ether causes a loss of hygroscopic properties with an approximate reduction of 0.03 per cent by weight. (The hygroscopic character of the material is due probably to the union of the ether-soluble fraction to the ether-insoluble portion.) The Kjeldahl Nitrogen present was 4.8 per cent.

The ether residue is gray in color, sticky, hygroscopic, fatty or waxy, gives none of the tests for protein, is soluble in acetone

* The tuberculin used in this work was prepared and donated by Eli Lilly and Company, Indianapolis, Indiana, through the courtesy and kind co-operation of the Biological Department.

² Löwenstein, E., and Pick, E. P., *Biochem. Z.*, 1911, xxxi, 145.

³ Gessard, C., and Vaudremer, A., *Compt. rend. Soc. de Biol.*, 1922, lxxxvii, 1012.

and in benzol, and is insoluble or only slightly soluble in chloroform. The nature of this substance and more detailed description of its properties are deferred for the present, pending completion of further studies. This derivative represents approximately 3.2 per cent of the original tuberculin by weight, and contains 0.0045 per cent Kjeldahl Nitrogen (not checked).

Positive skin tests were obtained by both Pirquet (cutaneous) and Manteau (intracutaneous) methods in dilutions ranging as high as 1:10,000,000. In view of the fact that these derivatives represent small fractions of the original tuberculin, their effective potency appears to be very great. The actual range of activity, therefore, was calculated between the limits of 1:72,000,000 and 1:333,000,000.

TABULATION OF QUALITATIVE TESTS.

	Alcohol Precipitate	Lecithin Derivative
Coagulability	Positive	Negative
Heller Test	"	"
Amm. Sulphate	"	"
Potass. Ferrocyanide	"	"
Picric Acid	"	"
Phosphotungstic Acid	"	"
Tannic Acid	"	"
Copper Sulphate	"	"
Mercuric Chloride	"	"
Silver Nitrate	"	Positive
Iodin Potass. Iodide	Negative	Negative
Mercuric Iodide	"	"
Xanthoproteic Test	Positive	"
Millon Test	"	"
Biuret Test	"	"
Adamkiewicz Test	"	"
Liebermann Test	"	"
Lead Acetate	—————	Positive
Molybdic Test	Positive	"
Lassaigne Test	"	"

The studies up to the present suggest that the antigenic radical of *B. tuberculosis* is contained probably in tuberculin derivatives such as these which have been described. That a specific substance is associated with the lipoids of *B. tuberculosis* has been shown by Boquet and Nègre,⁴ whose methyl alcohol extract of the organism conforms to the tuberculin derivative which we have described previously. Additional observations which appear to confirm our own studies may be found in those dealing with Wildbolz reaction. Von Bergen⁵ and Ichok⁶ found an alcohol-

⁴ Boquet, A., and Nègre, L., *Ibid*, 1922, lxxvi, 717.

⁵ von Bergen, J., *Schweiz. med. Wchnschr.*, 1921, xli, 655.

⁶ Ichok, M. G., *Ann. d. Med.*, 1921, ix, 97.

soluble antigen in the urine of tuberculous patients. Recently, Enright and Rettger⁷ reported on the chemical nature of the alcohol-soluble antigen in urine from tuberculous animals and patients, and characterized the substance as a phosphatid, probably lecithin, in accordance with its chemical properties.

⁷ Enright, J. J., and Rettger, L. F., *Am. Rev. Tuberc.*, 1924, x, 104.

MINNESOTA BRANCH

University of Minnesota Medical School, March 4, 1925.

170 (2693)

Differential absorption of anions by varieties of cotton.

By J. ARTHUR HARRIS, W. F. HOFFMAN and JOHN V. LAWRENCE.

[From the Department of Botany and the Division of Agricultural Biochemistry, University of Minnesota, Minneapolis, Minn., and the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.]

Earlier studies¹ have shown that the Egyptian and Upland types of cotton are differentiated with respect to certain physico-chemical properties of the leaf tissue fluids, such as osmotic concentration, specific electrical conductivity and hydrogen-ion concentration.² The higher specific electrical conductivity of the tissue fluids of the Egyptian, as compared with the Upland type of cotton, evidences for the absorption from the soil and the retention in solution of electrolytes as an important factor in the determination of the higher osmotic concentration of the Egyptian type. Analyses have shown³ that the chloride content is far higher in the Egyptian type.

We now note that these two types (closely enough related that at least first, second and third generation hybrids may be secured) are also characterized by differential absorption of sulphates.

Table I shows the mean chloride and sulphate contents of two series of about seventy analyses each on plants of Lone Star

¹ Harris, J. Arthur, Lawrence, Z. W., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T., *J. Agric. Res.*, 1924, xxvii, 267-328.

² Harris, J. Arthur, Hoffman, W. F., and Johnson, A. H., *Science*, N. S., 1925, lxi, 65.

³ Harris, J. Arthur, Lawrence, J. V., and Lawrence, Z. W., *J. Agric. Res.*, 1924, xxviii, 695-704.

TABLE I.
Comparison of Chloride and Sulphate content of leaf tissue fluids of Pima Egyptian and Lone Star Upland cotton as grown at Sacaton, Arizona, during 1923.

	N	Mean content for Egyptian Cotton	Mean content for Upland Cotton	Difference between Egyptian and Upland Cotton.	
				Absolute difference and probable error	Ratio of absolute difference to probable error
First series of determinations: July 29 to August 14. Grams chloride per liter Grams sulphate per liter	68	2.4412 \pm .0406	1.0515 \pm .0239	+1.3897 \pm .0396	35.1
	67	12.5373 \pm .1022	16.2164 \pm .0801	-3.6791 \pm .1410	26.1
Sec'd series of determinations: August 18 to August 31. Grams chloride per liter Grams sulphate per liter	68	3.3088 \pm .0477	1.1434 \pm .0302	+2.1654 \pm .0520	41.7
	70	14.1642 \pm .1294	17.3357 \pm .1793	-3.1714 \pm .1750	18.2
					+79.6 -25.6
					+97.3 -20.1

Upland and Pima Egyptian cotton growing in immediate association, and hence under identical environmental conditions.⁴ The values are given in terms of grams per liter. The probable errors of the means have been calculated with due regard to the correlation between associated plants due to the influence of environmental conditions.

The differences between both chloride and sulphate content in Egyptian and Upland cotton are many times (18.2 to 41.7) as large as their probable errors, thus leaving no possible doubt as to the differentiation of these two varieties with respect to their absorption of both of these anions.

The point to be emphasized here is that the behavior of these two types with respect to these anions is not merely quantitative in the sense that the tissue fluids of one of the two varieties contains larger quantities of electrolytes. It is differential in the sense that one variety contains large quantities of chlorides, whereas the other contains larger quantities of sulphates.

The differentiation in the reaction of the two genetically differentiated types may be most clearly brought out by expressing the differences in chloride and sulphate content as a percentage of the actual concentrations of these anions. Since the two types differ materially in the quantities of sulphates and chlorides, the results of taking either of the types as a base in the calculation of both percentage differences will be to some extent misleading. It seems best, therefore, to take the average of the constants for the two varieties as a base in the determination of the percentage.

The relative differences show that in chlorides the Egyptian type is from 80 to 97 per cent higher than the average of the two types, whereas the Upland type is from 20 to 26 per cent higher in sulphates.

The fertility of the hybrids between the two types affords the possibility of an investigation of the behavior of the differential reaction to these two anions in inheritance. This investigation is now under way.

⁴ Full experimental and analytical details are given in the papers cited above, and in another by Harris, Hoffman and Hoffman, *J. Agric. Res.* (in press).

171 (2694)

Empirical formulae for the proportionate growth of the human fetus.

By LEROY A. CALKINS and RICHARD E. SCAMMON.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

The following is a summary of a quantitative study of the growth of 70 external dimensions of the human body in the fetal period. Of these dimensions, 22 were of the head and neck, 28 of the trunk and pelvis, 16 were of the extremities, and 4 involved more than one major division of the body. Each dimension was determined from a series of preserved specimens, the number of cases ranging from 207 to 369.

Each dimension was plotted against the crown-heel or total body-length. In 19 instances the resulting curves approximated straight lines; in 41 instances the curves approximated straight lines, except at their upper ends. In 9 instances the relation could be approximated by two straight lines meeting in about the middle of the distribution. In one instance the relation in the lower ranges was expressed by a straight line, and in the upper by a curved one.

It was found that the departure from a straight line of the 41 curves mentioned above was due to the effects of birth moulding, to changes in the form of the chest following birth, and to formalin artifacts. Experimental studies were made of each of these factors. Corrections for head moulding were determined by the measurements of heads of children delivered by Cesarean section, and second twins born with breech presentation. The chest changes were determined by measurements of living newborn infants. The changes produced by formalin artifacts were also worked out quantitatively. The application of the corrections thus obtained to the upper values for the dimensions of this group reduced these curves to straight lines. The remaining 9 dimensions were mainly measurements in the anterior median line of the body. Their departure from straight lines is probably due to posture effects, but we have been unable to work out correction coefficients for them. Three remaining curves show

Anteroposterior diameter at umbilicus	208	0.165	-	1.0	0.164	-1.03	0.167	-1.89	3.68	5.22	3.77	5.19	3.75	5.15
Sternal notch to xiphi-sternal junction	233	0.11	-	1.5	0.113	-2.19	0.111	-1.84	0.71	3.79	0.65	3.76	0.66	3.70
Xiphi-sternal junction to pubis *	226	0.19 0.26	+ -	1.5 16.0	0.172 0.281	+3.62 -23.15	0.179 0.276	+2.58 -21.23	2.20	2.74	1.35	1.96	1.37	2.08
Pelvis height	245	0.155	-	4.0	0.153	-3.83	0.154	-4.15	0.67	2.46	0.56	2.00	0.53	2.07
Interspinoous diameter	245	0.145	-	2.5	0.147	-2.96	0.148	-3.20	0.77	1.48	0.78	1.68	0.81	2.01
Intercristal diameter	246	0.1625	-	2.5	0.166	-3.83	0.166	-3.65	1.10	2.54	0.86	2.08	0.88	2.06
Intra-trochanteric diameter	244	0.1775	-	3.5	0.186	-5.64	0.187	-5.91	1.50	2.68	1.39	3.30	1.45	3.62
Upper extremity length	314	0.40	-	4.0	0.40	-3.40	0.40	-3.35	1.12	1.20	1.15	1.45	1.16	1.11
Arm length	307	0.155	-	0.5	0.152	+0.27	0.152	+0.40	0.69	1.69	0.64	2.08	0.64	2.16
Forearm length	305	0.13	-	1.5	0.13	-0.50	0.13	-0.58	0.64	1.94	0.50	2.12	0.49	2.04
Hand length	304	0.12	-	3.0	0.12	-13.74	0.12	-3.35	0.51	2.44	0.43	2.35	0.57	1.94
Middle finger length	292	0.075	-	3.0	0.07	-2.76	0.07	-2.61	0.34	3.69	0.36	3.58	0.42	3.58
Arm circumference	294	0.195	-	8.0	0.193	-7.11	0.194	-7.49	1.87	3.26	1.88	2.96	1.87	2.84
Lower extremity length	315	0.43	-	7.0	0.429	-7.22	0.432	-8.29	1.28	1.62	1.33	1.49	1.04	1.13
Thigh length	301	0.19	-	2.0	0.19	-1.68	0.19	-1.65	0.63	1.30	0.60	1.39	0.61	1.41
Leg length	299	0.2	-	4.5	0.20	-3.34	0.20	-3.51	0.92	1.77	0.58	1.03	0.55	1.01
Foot height	298	0.04	-	1.5	0.04	-2.44	0.045	-2.68	0.54	5.72	0.41	5.66	0.48	6.93
Foot length	299	0.16	-	6.5	0.16	-6.11	0.156	-5.53	0.56	3.88	0.61	3.15	0.61	3.19
Thigh circumference	294	0.31	-	13.0	0.31	-13.76	0.314	-14.26	2.91	3.12	3.05	3.65	3.11	3.98

* Upper constants for use between 5 and 30 cm. crown-heel length; lower constants for use between 30 and 35 cm. crown-heel length.

deviations which are probably due to faulty technique and to the combination of several experimental errors.

Since all of the dimensions which we have been able to analyze in detail approximate straight lines, when plotted against total body length, they may be expressed by formulæ of the general type:

$$D = aL \pm b$$

where "D" is the dimension in question, "L" is the total body-length, "a" is a constant in the form of a decimal fraction and "b" is a second constant. These constants have been determined by graphic methods and by the methods of averages and of least squares, on the basis of the means for dimension length and total body-length for the 5 cm. intervals of total body-length between 5 and 55 cm. inclusive. The preceding table gives constants for the formulæ for 33 of the more important dimensions of the series as determined by these methods. The columns forming the left division of the table give the average weighted absolute and percentage deviations (summed without regard to sign) of the observed from the calculated 5 cm. range averages. The constants as given are for use with dimensions taken in millimeters.¹

Since all of the dimensions of the body which we were able to analyze in detail are of the straight line type, it follows that the growth in length, girth and diameter of the various external divisions of the body is directly proportional to the growth in total body-length in the fetal period (from at least 3 fetal months to birth). In other words, while each dimension has its own rate of growth with respect to body-length, this rate does not change in the period under consideration.

When the formulæ are grouped according to regions, it is found that practically all head and neck measurements have a positive second or "b" constant. The "b" constants for the formulæ for the chest dimensions may be zero, or small positive or negative ones. The "b" constants for the formulæ for the abdomen and pelvis and extremities are, with one exception (arm length by least squares), negative. Since the "b" constant is an indicator of the amount of growth prior to the period under con-

¹ A large number of empirical formulæ for the obstetrical dimensions of the head have been published elsewhere. (Calkins, *Am. J. Obstet. and Gyn.*, 1922, iv, 109.)

sideration, it is evident that the head and neck have grown more proportionately than the body as a whole in the embryonic period, while the thorax has undergone about the same relative amount of growth as the body as a whole. The lower part of the abdomen, the pelvis and the extremities have grown relatively less. This forms a quantitative demonstration of the application of the law of developmental direction to the growth of the human body in prenatal life.

172 (2695)

The antigenic properties of pneumococci and streptococci treated with sodium ricinoleate.

By W. P. LARSON and EDMOND NELSON.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

The property of sodium ricinoleate to neutralize bacterial toxins and destroy the pathogenicity of some of the pathogenic bacteria has been emphasized in a series of papers published from this laboratory.^{1, 2, 3} The present paper concerns the effect of sodium ricinoleate upon the pathogenic and antigenic properties of the pneumococcus and streptococcus scarlatinæ.

If a solution of sodium ricinoleate is added to a virulent culture of the pneumococcus, so that the final dilution of soap is 0.1 per cent, the micro-organism loses its pathogenicity instantly. Ten cc. or more of such a culture may be injected into rabbits intraperitoneally without ill effects. Twenty-four hours after such an injection, large amounts of agglutinins are present in the blood stream. Following such treatment, the animals resist many lethal doses of pneumococci. The serum of rabbits thus immunized protects normal rabbits against intraperitoneal and intravenous infections.

We have studied the effect of sodium ricinoleate upon one strain of streptococcus scarlatinæ. It loses its power to grow upon culture media in less than five minutes when treated with

¹ PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 229.

² Ibid., 1924, xxi, 278.

³ Ibid., 1924, xxii, 194.

0.5 per cent sodium ricinoleate. Streptococci so treated with soap produce agglutinins in rabbits within twenty-four hours following intraperitoneal injection.

173 (2696)

Observations on the measurement of the pH of soap solutions.

By H. O. HALVARSON. (Introduced by W. P. Larson).

[From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.]

Coincident with Larson's studies¹ on the neutralization of toxins with sodium ricinoleate, studies on the chemical properties of this soap have been conducted. One phase of this work has concerned itself with methods of determining the pH of solutions of this compound. In this work certain important anomalies in the measurement of pH of soap solutions have been observed which are here reported.

All measurements were made with a Leeds and Northrup, Type K. potentiometer, using a .1N Calomel electrode. To investigate the problem thoroughly it was decided to carry out measurements with a series of electrodes instead of relying upon the results obtained from a single one. In all cases the various electrodes were tested with a sodium borate, boric acid buffer. Only those were used that gave correct readings on this buffer solution. From the following table it will be seen that electrodes which gave check readings on the buffer solution would not give check readings on the soap solution:

TABLE NO. 1.

Measurement of pH of a 5 per cent Solution of Sodium Ricinoleate using the Bailey Electrode.²

Electrode No.	pH of Buffer before making readings on Soap	pH of Soap Solution			pH of Buffer after making readings on Soap
		1st reading	2nd reading	3rd reading	
1	8.99	6.99	7.01	6.96	8.99
2	8.99	7.64	7.62	7.65	8.99
3	8.99	7.92	7.90	7.96	8.99
4	8.99	7.98	7.96	8.00	8.99

¹ See Page 357.

² *J. Am. Chem. Soc.*, xlii, 45.

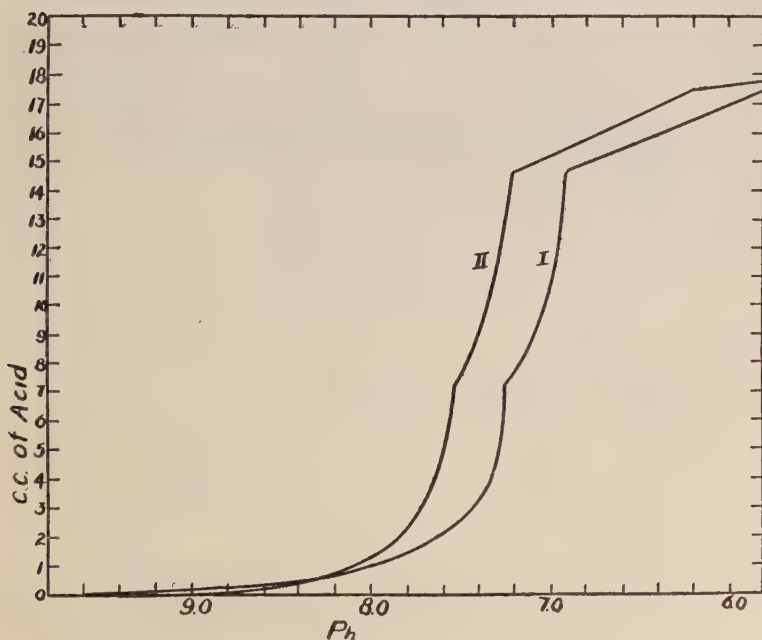
Believing that these anomalies were due to a surface phenomenon, electrode No. 1 was replatinized and then used. The results are given below:

TABLE NO. 2.
Effect of Replatinizing upon the pH reading on a 5 per cent solution of Sodium Ricinoleate.

	pH of Buffer	pH of Soap Solution	
		1st reading	2nd Reading
Before replatinizing	8.99	6.95	6.97
After replatinizing	8.99	7.45	7.40

That each electrode gives fairly consistent results, but that these results differ considerably from those obtained when the electrode is replatinized, is shown by the following graphs. In these graphs the number of cc. of 1/14 N H Cl added to a 5 per cent solution of sodium ricinoleate is plotted against the pH as measured by the electrode after the addition of each cc. of acid. Curve No. 1 was obtained by using the electrode before it was replatinized, and curve No. 2 was obtained by using the electrode after it was replatinized.

A modification of the Hildebrand bubbling electrode gave re-



sults almost as variable. A series of these electrodes were used. The results are given in the following table :

TABLE NO. 3.
pH values obtained on a 5 per cent solution of Sodium Ricinoleate using the bubbling electrode.

Electrode Number	pH of Buffer before measuring the soap	pH of Soap	pH of Buffer after measuring the soap
1	8.99	9.01	8.99
2	8.99	9.03	8.99
3	8.99	9.13	8.99
4	8.99	8.94	8.99
5	8.98	9.08	8.98

It appeared that the electrode could not reach the correct equilibrium; so measurements were made on a solution of a saturated soap. The sodium pelargonate was used, as the higher soaps foamed so much as to interfere with the equilibrium. Readings were taken while the hydrogen was bubbling, and also while the hydrogen was shut off :

The following table gives the results :

TABLE NO. 4.
pH values obtained on a 5 per cent solution of Sodium Pelargonate using the bubbling electrode.

Electrode No.	Buffer before measuring soap		Soap		Buffer after measuring soap	
	Hydrogen Bubbling	Hydrogen Shut off	Hydrogen Bubbling	Hydrogen Shut off	Hydrogen Bubbling	Hydrogen Shut off
1	8.99	8.99	8.86	8.86	8.99	8.99
2	8.99	8.99	8.87	8.87	8.99	8.99
3	8.99	8.99	8.87	8.86	8.99	8.99
4	8.99	8.99	8.86	8.86	8.99	8.99

Using Sodium Bianoleate.

Electrode No.	Buffer before measuring soap		Soap			
	Hydrogen Bubbling	Hydrogen Shut off	Hydrogen Bubbling	Hydrogen 1st r'd'g	Shut off 1'*	Shut off 5'*
1	8.99	8.99	8.83	8.45	8.18	8.03
4	8.99	8.99	8.96	8.66	8.39	8.23

*After 1 min., after 5 min.

It appears from these measurements that the platinized electrode acts as a reducing catalyst for the reduction of the sodium ricinoleate which brings about an equilibrium that depends upon

the nature of the surface. With the Bailey electrode this equilibrium had been reached before a reading could be made. Since two electrodes with the same surface cannot be made, it is impossible to make two electrodes that will give check readings on the sodium ricinoleate solutions. Neither is it likely that an electrode will give the same results after being replatinized.

One must also conclude from this work that, because an electrode gives correct readings on a buffer, it does not necessarily mean that it will also give the correct reading on any other non-poisonous solution.

SCIENTIFIC PROCEEDINGS.

NEW YORK MEETING

College of the City of New York, April 15, 1925.

174 (2697)

Preventing glucolysis in blood samples.

By ANTON R. ROSE and FRED SCHATTNER.

*[From the Laboratory of the Prudential Insurance Company,
Newark, N. J.]*

When blood samples are set aside at room temperature with no preservative, the sugar content as measured by the reduction of picric acid diminishes markedly. This is most pronounced on the first day, and continues, until in the course of two to four days the glucose may have entirely disappeared. Commercial glucose added to the sample is similarly destroyed. If the samples are not chilled or treated chemically, sugar determinations are of little value unless the analyses are made immediately. It is not always practicable to make determinations promptly, nor can the samples always be kept on ice in the interim. An inquiry was therefore made to find practical means of checking, or if possible, completely preventing glucolysis.

Blood preservatives have been recommended in various scientific journals¹ but these were found to be unsatisfactory, and a systematic investigation was therefore undertaken.

The work has been done on the blood of herbivorous animals with a large number of substances, and is now being continued

¹ Major, R. H., *J. Am. Med. Assn.*, 1923, lxxxi, 1952; Denis, W., and Beven, J. L., *J. Lab. Clin. Med.*, 1924, 9x, 674; Sander, F. V., *J. Biol. Chem.*, 1923, lviii, 1; Denis, W., and Aldrich, M., *J. Biol. Chem.*, 1924, xlv, 203.

on human specimens. The fluorides recommended by other workers¹ were first tested. These inhibit glucolysis, and in most human specimens keep the blood sugar nearly constant for several days, but can not be relied on for all specimens over the 10 days required as a minimum by the Prudential Laboratory. In addition to the fluorides a large number of substances varying widely in their properties have been tried. Eventually the work has narrowed down to halogen derivatives of hydro-carbons. It was found that trichlor-ethelene mixed with NaF is fully as effective as thymol and NaF. Neither $\text{CCl}_2=\text{CHCl}$ nor NaF alone are of any value. Trichlor-methane and tetra-chlor-methane were soon discarded as worthless for our purposes. Chlor-benzol and brom-benzol keep the sugar content of blood samples near its initial value for days, and in combination with NaF have so far proven the best.

Increasing the halogen on the benzene ring diminished its anti-glucolytic properties. Between chlor and brom derivatives of benzol no difference could be noted, but iodo-derivatives proved less useful as preservatives.

Introducing aliphatic side chains into the ring gave less effective preservation.

In case of the sheep's blood which has been so far mostly used in these studies, no combination has yet been found which will prevent a fluctuation of 0.01 per cent glucose in the samples during the first three days. These fluctuations are either a drop the first day with a rise on the second day, or, less frequently, a rise followed by a decrease in sugar. After the fourth day there is very little change when a halogen benzene compound is present in the blood sample, but the non-halogen preservatives allow the amount of sugar to drop very abruptly on the third day, becoming a mere trace on the fourth or fifth day. In a large number of cases where the halogen-derivative fluoride combination has been used, the glucose at the end of 15 to 20 days is within 0.015 per cent of the initial value. Two samples were kept for 72 days with a final discrepancy of less than 0.015 per cent, whereas the same blood treated with NaF and thymol lost half its glucose in three days, and all of it in six days.

175 (2698)

The factors of dehydration in rabbits following pyloric obstruction.

By JAMES L. GAMBLE and MUNROE A. McIVER.

[From the Departments of Pediatrics and of Surgery of the Harvard Medical School, Boston, Mass.]

Data obtained from rabbits consist of measurements of water, chlorides, and fixed base in the gastric contents of controls and after obstruction of the pylorus. They show (Tables 1 and 2), following obstruction, a loss of water, chlorides and fixed base into the stomach of from 2 to 3 times an estimated initial total plasma content.

A chief point of these findings is the large loss of fixed base. From the point of view of repair of dehydration following pyloric obstruction, the loss of base is more significant than the loss of chloride ion, for the reason that it represents an absolute depletion of the body's content of dissolved electrolytes, whereas loss of chloride ion is replaced by bicarbonate ion. Replacement of base is thus indicated as the essential factor in the beneficial action of injections of NaCl solution in the presence of pyloric obstruction.

Haden and Orr,¹ however, regard the action of NaCl solution as protective against a toxic substance rather than simply reparative of dehydration. They state that the chloride lowering in the plasma following pyloric obstruction can only in part be explained by a loss in gastric secretion, since they find that it occurs when there is little vomiting and in rabbits which cannot vomit. They suggest that chloride leaves the plasma in offensive quest of a toxic substance. The data here given demonstrate that although these rabbits did not vomit, they nevertheless lost into their stomachs several times the total plasma capacity for chloride. That any chloride at all is found in the plasma proves a movement in the direction opposite from that surmised by Haden and Orr. The chloride lowering in the plasma is thus, as might be expected, shown to be referable to circumstances affecting body fluid adjustments.

¹ Haden, R. L., and Orr, T. G., *J. Am. Med. Assn.*, 1924, lxxxii, 1515.

TABLE I.

	Animal No.	Body Wt.	Stomach Contents				Blood Serum.	
			Solids.	Water.	Fixed Base.	Chlorides.	Fixed Base.	Chlorides.
		Kilos	gm.	cc.	cc. 0.1N	cc. 0.1N	cc. 0.1N per 100	cc. 0.1N per 100
Controls	1	2.4	21	89	68	155	169	100
	2	4.0	34	98	116	196	172	105
	3	2.8	20	67	64	100	167	107
Average		3.1	27	85	83	150	169	104
Pylorus obstructed*	4	3.0	17	313	376	462	-----	62
	5	2.7	33	285	388	410	156	78
	6	3.8	21	320	327	540	-----	---
Average		3.3	24	306	364	471	156	70

*Survived operation 20-28 hours.

TABLE II.

Estimation of *plasma volume* and of total plasma content of Cl and B compared with loss of H₂O and of Cl and B in gastric secretion following pyloric obstruction.

Animal	No. 4	No. 5	No. 6
Plasma volume ¹	93 cc.	84 cc.	118 cc.
Water lost ²	228 cc.	200 cc.	235 cc.
Water lost÷Plas. vol.	2.5	2.4	2.0
Total plasma chloride ³	96 cc. 0.1N	87 cc. 0.1N	123 cc. 0.1N
Chloride lost ²	312 cc. 0.1N	260 cc. 0.1N	390 cc. 0.1N
Cl lost÷Total plas. Cl	3.4	3.1	3.2
Total plasma base ⁴	157 cc. 0.1N	142 cc. 0.1N	200 cc. 0.1N
Base lost ²	293 cc. 0.1N	305 cc. 0.1N	244 cc. 0.1N
B lost÷Total plas. B	1.9	2.3	1.2

¹ Body wt. \times 0.031 (according to Uthelm, K., *Am. J. Dis. Child.*, 1920, xx, 366).

² By subtracting average for controls from total measurement (see Table I).

³ Plasma volume \times 1.04 (see Table I).

⁴ Plasma volume \times 1.69 (see Table I).

176 (2699)

Perfusion studies on pancreas and liver.

By WILLIAM S. COLLENS. (Introduced by Graham Lusk).

[From the Physiological Laboratory of the Cornell University
Medical College, New York City.]

Because of the rich anastomotic supply to the pancreas, duodenum and liver, an anatomical study was undertaken with the view of determining what arteries go directly to each of these organs, so that a more exact knowledge of the direction taken by any perfused fluids could be established. A modification of Gross's^{1, 2, 3} method of injecting the coronary vessels of the heart was used, and the tissues cleared by the Spalteholz method. A description of the method of preparation of these specimens will follow in a later paper. Proper temperature and pressure controls were used in order to approximate normal conditions in living organism.

X-ray photographs reveal that the hepatic artery coming from the coeliac axis gives off the right gastric and the large superior pancreatico-duodenal. This latter artery supplies the neck and body of the pancreas and the first portion of the duodenum. Two small branch arteries, a continuation of the hepatic, ascend to furnish the arterial supply to the liver. The inferior pancreatico-duodenal artery arises from the superior mesenteric artery, and supplies the head of the pancreas and the second portion of the duodenum. A rich anastomosis can be seen in both the duodenum and pancreas between the superior and inferior pancreatico-duodenal arteries. The pancreas is also visualized by this method. The body and tail of the pancreas, on dissection of the specimens, are seen to be supplied by several small branches arising from the splenic artery. The veins draining the pancreas all enter the portal system. One may notice, then, that the arteries furnishing the most practical approach for direct perfusion of the pancreas are the superior and inferior pancreatico-duodenal arteries.

¹ Gross, L., *The Blood Supply to the Heart*, Paul Hoeber, New York, 1921, pp. 5-8.

² Benzley, R. R., *Am. J. Anat.*, 1911, xii, 297.

³ Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 194.

Perfusion experiments of these arteries were then undertaken to determine their effect upon the blood sugar and sugar excretion in the urine. The animals used were dogs. The anesthetic was amytal in doses of 0.6 cc. of a 10 per cent solution per kilo of body weight. The Shaffer-Hartmann method of blood sugar determination was employed, and the Benedict test for the urine. Ten cc. of normal saline at 40° C. were used in all the perfusion experiments.

Epstein,⁴ in an extensive series of experiments, has found that when he perfused the arterial blood supply to the pancreas, hyperglycemia and glycosuria constantly occurred. He interpreted these experimental results as indicating that the process of perfusion in some way caused trypsin formed in the pancreas to inactivate insulin, with consequent production of the diabetic condition. He concluded that this reaction between trypsin and insulin constituted an important etiological factor in the production of diabetes.

Repetition of these experiments of Epstein by the perfusion of saline into the hepatic artery of the dog confirmed his original observations; but when the two terminal hepatic arteries were ligated the pancreas could be perfused without causing either hyperglycemia or glycosuria. On the contrary, when the superior pancreatico-duodenal artery was ligated, with the hepatic branches patent, perfusion resulted in both hyperglycemia and glycosuria. This clearly indicates that the effect is upon the liver and not upon the pancreas.

Furthermore, perfusion of the inferior pancreatico-duodenal artery, which has no relation to the arterial supply of the liver, has no effect whatever upon the blood sugar, and produces no glycosuria.

If saline be introduced directly into the portal vein there appears inconstantly a delayed (one-half to one hour) excretion of traces of sugar in the urine, with no or only a very slight elevation of blood sugar. It appears from this that glycogenolysis is more readily induced through the hepatic artery than through the portal blood stream.

⁴ Epstein, A. A., and Rosenthal, N., E. H. Maeckling and V. de Beck, *Am. J. Physiol.*, 1924, lxx, 225; *Ibid.*, 1925, lxxi, 316.

177 (2700)

Anhydremia with insulin and water intake.

By DAVID L. DRABKIN* and H. SHILKRET. (Introduced by
Lafayette B. Mendel).

[*From the Laboratory of Physiological Chemistry, Yale University,
New Haven, Connecticut.*]

Pronounced hypoglycemia due to the injection of large amounts of insulin has been shown by Drabkin and Edwards¹ to be associated with a concentration of the blood. Although this symptom was invariably found, the animals studied fell into two groups: some developed anhydremia rapidly with an acute fall in blood pressure; a larger group exhibited a gradual rise in blood concentration with a well-maintained blood pressure. The clinical observations of Joslin, Gray and Root² indicate that desiccation through diarrhea renders patients prone to dangerous insulin hypoglycemia.

The natural outgrowth of the problem was to study the influence of the water intake upon the type of reaction with insulin, especially with reference to the severity of the resulting anhydremia. A more exact statement of the problem was framed in the question: "Will an animal which has been desiccated through water starvation react differently to a large amount of insulin than one which has had plenty of water?"

The procedure was as follows: Unanesthetized dogs (5 to 10 kilograms in weight) were used. The first "control" blood samples were drawn, and the dogs were either thirsted (water starved) or given by stomach-tube an average of 800 cc. of water daily for periods of 3 to 7 days. As an added check upon the results, some of the dogs previously thirsted were also studied under a forced water régime, and vice versa. Just before the administration subcutaneously or intravenously of a large dose of insulin (20 units per kilogram body weight), the second "control" samples were drawn. Blood samples were then taken at suitable intervals following the insulin administration. Each blood sample was analyzed for hemoglobin and dry solids, as

* Medical Fellow of the National Research Council.

¹ Drabkin, D. L., and Edwards, D. J., *Am. J. Physiol.*, 1924, lxx, 273.

² Joslin, E. P., Gray, H., and Root, H. F., *J. Metab. Research*, 1922, ii, 651.

indices of the amount of blood dehydration, and for sugar. A modified Cohen and Smith³ technique was employed for the estimation of the hemoglobin, and the method of Schaffer and Hartmann⁴ was used for the determination of the sugar. The physical qualities of the blood samples were also noted. A careful record was kept of the symptomatic responses and their nature. Under this head were noted such factors as the presence or absence, type and the time of onset of convulsions, the time of onset of coma, the rapidity and type of recovery if any after the administration, in each case, of resuscitative amounts of glucose (150 to 200 cc. of a 10 per cent solution intraperitoneally).

The experimental results may be summarized as follows: Water starvation for 4 days caused a negligible increase in the concentration of the blood. Thirsting the dogs for longer periods of 5 to 7 days, however, resulted in a moderate concentration (12.8 to 17.4 per cent above the original values).

The response of the blood sugar to insulin was the same in the desiccated dogs as in those which had received water. In each group the fall was rapid and to a low level (from the original values to 30 mg.—0.00 mg. per 100 cc. of blood).

The desiccated animals developed a more profound concentration of the blood than the others. The blood concentration of the water-starved dogs was 36.9 to 64.3 per cent (as determined from the hemoglobin values), 26.9 to 33.5 per cent (as determined by the dry blood solids) in comparison with the corresponding values of 16.7 to 28.0 per cent and 9.7 per cent in the non-desiccated dogs. A graphic record of the hemoglobin concentrations of the two groups showed interesting differences. The curve of concentration of the animals which had no water was steep and continuous; that of the others was more gradual in its ascent, with a tendency for spontaneous return to the normal value during the third hour.

The dogs which had been given water immediately recovered upon the administration of glucose and stayed well. The desiccated animals came out of their coma after the administration of glucose, but were extremely weak, none surviving more than 24 hours. The production of anhydremia on top of desiccation is apparently incompatible with the life of the cells.

³ Cohen, B., and Smith, A. H., *J. Biol. Chem.*, 1919, xxxix, 489.

⁴ Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920-21, xlv, 365.

The most striking observation, however, was the absence of convulsions in the desiccated dogs, and the presence of severe, spastic convulsions in those with water.

Conclusions:

Dogs, desiccated through water-starvation, when given large doses of insulin developed a more severe anhydremia than the dogs which had water.

Convulsions were not observed in the desiccated animals although they invariably appeared in the others.

Glucose did not prevent the death of the water-starved dogs. The non-desiccated animals immediately recovered after glucose administration.

178 (2701)

The emetic action of strophanthidin in cats with denervated hearts.

By M. DRESBACH and K. C. WADDELL.

[From the Physiological Laboratory, Albany Medical College, Albany, N. Y.]

Strophanthidin is a cleavage product derived by hydrolising hispidus or Kombé strophanthin. It can also be obtained from cymarín, an active principle of *Apocynum cannabinum*.¹ Its empirical formula,² as lately established, is $C_{23}H_{32}O_6 \cdot \frac{1}{2} H_2O$. Jacobs and Collins have, moreover, thrown much light upon its structural makeup.³ Pharmacologically, strophanthidin has been but little studied.

As we have already pointed out,⁴ this substance has marked emetic properties. It also acts upon the heart in a manner essen-

¹ Windaus and Hermanns, *Ber. der deutsch. chem. Ges.*, 1915, xlviii, 979, 991.

² Jacobs and Heidelberger, *J. Biol. Chem.*, 1922, liv, 253; Thoms and Unger, *Zeit. f. angewand. Chem.*, 1924, xxxvii, 721.

³ Jacobs, *J. Biol. Chem.*, 1923, lvii, 553, 569; Jacobs and Collins, *ibid.*, 1924, lix, 713, and lxiii, 123.

⁴ Dresbach and Waddell, *J. Pharm. and Exp. Therap.*, 1924, xxiii, 152.

tially like that of the strophanthins from which it is derived, when given in suitable doses.

Since strophanthidin is either quite rapidly eliminated from, or destroyed in, the animal body, its emetic action can be advantageously observed repeatedly on the same individual animal. For this purpose, we have used cats (and a few dogs). We have found that emesis occurs promptly after any of the usual modes of injection (except, perhaps, by mouth), but we have employed the intraperitoneal route for the most part, confirming the results by intravenous injections. The average intraperitoneal dose is 0.20 mg. per kg.

The research here reported deals mainly with emesis in cats with partially and completely denervated hearts, though we have previously tested the substance on a large number (more than sixty) of normal cats. The operations performed and the number of animals used were as follows: 13 bilateral stellate ganglion excisions; 7 unilateral vagotomies, 2 double vagotomies; 12 double vagotomies with bilateral stellate ganglion excisions (extrapleural method); 9 bilateral vagotomies with bilateral (intra-thoracic) stellate ganglion excision, together with removal of most of the thoracic sympathetic chain; 5 additional operations of this sort, with removal of the middle cervical ganglia also, thus insuring absolute denervation of the heart; 3 spinal cord sections at level of 7th vertebra; 3 decerebrations.

In the work recently done in order to insure complete denervation of the heart, we have carried out the operations in several stages and have had the animals in good condition, a few being able to eat and retain food after the final vagus section. With one exception, in which only nausea was produced, all of the cats in the above series vomited after injections of strophanthidin intraperitoneally, and in those cases in which intravenous injections could be used the results were also positive. The cats with spinal cord sections were no exceptions, though this operation seriously interferes with the vomiting act. Nevertheless, it rules out the probability of strophanthidin acting on any visceral end organ to produce reflex vomiting.

We conclude that this substance does not bring about emesis by acting on some afferent structure in the heart, or at least not wholly in this way. In the light of our experiments its seat of action is more probably the vomiting center. Other phases of the action of strophanthidin are being investigated.

"Residue antigen" from a strain of Friedlander bacillus.

By J. HOWARD MUELLER, DOROTHEA E. SMITH and STELLA
LITARCZEK.

[From the Department of Bacteriology and Immunology, Harvard
University Medical School, Cambridge, Mass.]

The work of Zinsser and Parker,¹ Heidelberger and Avery² and one of us (Mueller)³ has indicated that most bacteria produce carbohydrate gums possessing the property of reacting specifically with antibodies formed in response to injections of the original bacteria. It is, therefore, of interest to study a number of these gums, called by Zinsser "residue antigens," from different bacterial sources. Only by such study can it be determined to what extent it will be necessary to alter the present conception of the chemical basis of immunological specificity.

Since the Friedlander Bacillus grows readily and abundantly on simple media, and is known to produce considerable quantities of a complex carbohydrate presumably related to the capsular material,⁴ * this organism was chosen as being particularly adapted to the work.

An old stock strain has been used, the source of which is unknown. It may be mentioned here that among several strains examined, there are distinct serological differences, as might be expected from the unsatisfactory cultural classification of this group. Work upon other strains is being continued by one of us, (Smith) and it is possible that a classification of the group based on relationship of the residue antigens produced may be developed as an incident to the purely chemical study of these substances.

¹ Zinsser and Parker, *J. Exp. Med.*, 1923, xxxvii, 275.

² Heidelberger and Avery, *J. Exp. Med.*, 1923, xxxviii, 73, and 1924, xl, 301.

³ Mueller, *Proc. Soc. Exp. Biol. and Med.*, 1925, xxii, 209.

⁴ Toeniessen, *Centralbl. f. Bakt. I Abt. Orig.*, 1920-1, lxxxv, 225.

* Since this article was written, we have found that by following exactly Toeniessen's technic for the preparation of "capsular material" from cultures grown on ordinary agar in Petri dishes, a substance is obtained which precipitates with immune serum more rapidly at a dilution of 1-1,000,000 than the material obtained from broth cultures. The yield is somewhat greater, and the preparation is much simpler. Not enough of this substance is available for analyses, but it is apparently similar in being largely carbohydrate.

Cultures were made in broth, prepared from casein hydrolyzed for six hours with four times the weight of hydrochloric acid. After removal of the excess acid, and neutralization, there is added an inorganic salt mixture and glucose. One hundred liters of broth were used for the preparation here reported. After a week's growth in the incubator, the flasks were Arnolded for one hour, passed through a Sharples centrifuge to remove the bacteria, concentrated to about one tenth on the water bath and precipitated by adding two volumes of alcohol. Upon centrifuging, a two-layer precipitate forms, the lower layer being a brown syrup, the middle layer a grayish white solid cake. Most of the residue antigen is in the lower layer. This material, after reprecipitating with alcohol, dialyzing and again precipitating with alcohol, yields about 25 grams of material, giving a ring with immune rabbit serum at 1-100,000. It is purified further by dissolving in water, and acidifying with hydrochloric acid until no more precipitate separates. This is removed by centrifugation and proves to be relatively inactive with serum. By the addition of about one and one half volumes of glacial acetic acid to the somewhat turbid supernatant fluid, another bulky precipitate separates and is centrifuged off. This also is mostly impurities. To the supernatant, more glacial acetic acid is added, to make a total of about two volumes. A heavy, curdy yellowish white precipitate separates quite sharply, which, after reprecipitating once in the same way with acetic acid, is washed with alcohol and ether and dried. The yield is about 3.0 grams.

It does not dissolve readily in water but forms a very turbid opalescent suspension, which clears quickly with a small quantity of NaOH solution, giving a yellowish solution. It yields a ring with immune serum at 1-1,000,000. The nitrogen content is 1.3 per cent, and there is an appreciable quantity of phosphorus present, but no sulfur. Reducing sugars are formed in considerable quantity on acid hydrolysis, and from these an osazone has been obtained which has not yet been definitely identified.

A further comparative study of the chemical composition of this substance, and of similar substances isolated from other strains of Friedlander bacilli, is being made.

180 (2703)

On the mechanism of insulin action.

By ERNST FRIEDRICH MUELLER, HERBERT J. WIENER and
RENEE von E. WIENER.

[*From the Department of Metabolism, Vanderbilt Clinic, College of Physicians and Surgeons, New York City.*]

The generally accepted explanation of the blood sugar lowering effect of insulin when injected into the mammalian organism, either subcutaneously or intravenously, is that an interaction takes place between the injected hormone and the cells or tissue fluids of the body. The chemical structure of insulin is unknown, and the nature of its chemical reactions within the body has not been determined up to the present time.

Mueller and Corbitt¹ found that in rabbits there was a difference in the blood sugar lowering effect between the intradermal and the subcutaneous administration of insulin. By intradermal injection the blood sugar lowering effect was found to be of longer duration than by subcutaneous injection.

This observation led to further research work upon the effect of the two methods of administration of insulin in human subjects. The results form the basis of the present communication. Diabetics as well as individuals with a normal carbohydrate metabolism were studied. The experiments were done in the morning, with the subject in a fasting condition, or following a breakfast of definite composition, finished at a known time. For the injections the U 40 strength of Insulin Lilly (Iletin) was employed. Two experiments on the same individual, employing like doses of insulin from the same bottle and under the same dietary conditions, but differing in the method of administration of the insulin, constitute a series and are so referred to in the text to follow. A total of 33 such series are here reported.

The blood sugar was determined immediately before the insulin injection, and at one hour, two hours and four hours following the injection. In a sufficient number of cases the same blood sugar curve, but omitting the insulin injection, was determined.

¹ Mueller and Corbitt, *J. Lab. and Clin. Med.*, 1924, ix, 3.

In six additional series of one hour only, the blood sugars were determined at twenty minute intervals following the injections.

Twenty-nine (88 per cent) of the thirty-three series show a greater reduction in the blood sugar level at the first hour following the intradermal injection than following a subcutaneous injection of an equal dose.

At the second hour, twenty-three (70 per cent) of the intradermal injections resulted in a lower blood sugar level than did the subdermal injections.

At the fourth hour, twenty, or 67 per cent, of the series showed a greater hypoglycemia with intradermal administration.

The difference in the response to the two methods of administration, as manifested by the hypoglycemic effect, is most striking with the smallest doses, (*i. e.*, 5 units) and at the first hour after the injection.

This difference in response to the two methods of administration varies from case to case. The greatest differences in the hypoglycemic effect between the intradermal and the subdermal methods occur in the cases of moderately severe diabetes. The least differences are noticeable in non-diabetics. A consistently less great response to the intradermal injection was noted in only one case of severe diabetes. The figures show characteristic curves obtained by plotting the blood sugar values, following the insulin injection, expressed in per cent of the initial blood sugar before the injection, against time. Figure IV represents the composite curves of all the 5 and 10 unit series. The fact that the maximum difference falls at the first hour, and the gradual decrease in this difference at the subsequent time intervals, is shown in this curve.

Insulin injected subcutaneously enters the blood stream and is brought to the tissues more promptly than when the same dose is given between the layers of the skin. Kasahara² showed that the resorption of true solutions, colloidal solutions and cell and bacterial suspensions when introduced intracutaneously is materially delayed as compared to identical injections administered subcutaneously. Our results cannot therefore be explained by a quicker resorption of the intradermally introduced insulin. From the 20-minute series (Fig. V) it is apparent that there is a blood

² Kasahara, *Ztschr. f. d. ges. exp. Med.*, 1925, xliv, 294.

sugar lowering effect of intradermally injected insulin before any of the hormone can possibly be present in the blood stream, for the subdermally administered equal dose of insulin shows no such effect twenty minutes after the administration. We conclude from the observations reported above that insulin injected intracutaneously must have an initial specific action produced in some way other than the usual contact of the hormone with the tissue fluids and cells.

Our findings imply the transmission of a specific stimulus between the intracutaneous area of the skin and the organ or organs the activity of which governs the carbohydrate metabolism in the mammalian body. We postulate the pathway which carries this stimulus to be the fibres of the parasympathetic nervous system. Claude Bernard showed that stimulation of certain parts of the parasympathetic system results in increased glucose formation in the body. M. Eiger,³ working with turtles showed that stimulation of the peripheral end of the cut vagus caused increased glycogenesis, despite exclusion of the pancreatic activity. H. Meyer showed that injection of pancreatic extract inhibits glycogenolysis in the liver. From these experiments it is to be assumed that stimuli may travel via the vagus nerve to the liver, and there cause actual increased liver cell metabolism, and also that such stimuli may travel to the pancreas whereby an equivalent internal secretory influence of the liver cell metabolism may be registered. In both cases vagus stimulation results in the inhibition of glycogenolysis.⁴ Minkowski's experiments have shown that not only extirpation of the pancreas, but also disconnection of the nerves between pancreas, liver and duodenum results in diabetic symptoms.

The response of the liver cell in the form of glycogenetic activity is governed to a large extent by the amount of glycogen in the liver at the time, and this response will be decreased if the liver is rich in glycogen. The blood sugar levels following the injections of 10 and 20 unit doses of insulin intradermally as well as subcutaneously on two non-diabetics are shown in Figure II. The quick recovery to the normal blood sugar level in both, as well as the reversed response of one of the subjects to the experiment, is probably due to the degree of glycogen saturation of the

³ Eiger, M., *Centralbl. f. Physiol.*, xxx:

⁴ Quoted from L. R. Mueller, *Die Lebensnerven*, Berlin, Springer, 1924, 282.

liver cell. Experiments are now being done in the attempt to prove that the effect of an intracutaneous injection of insulin is greatest when the liver is poor in glycogen, and that with the liver rich in glycogen, in the same individual, the effect of a subdermal injection will be as great, or greater than that of a like dose injected intradermally.

The glucolysis occurring in the bloods drawn before insulin injection and at stated intervals thereafter was determined in nine

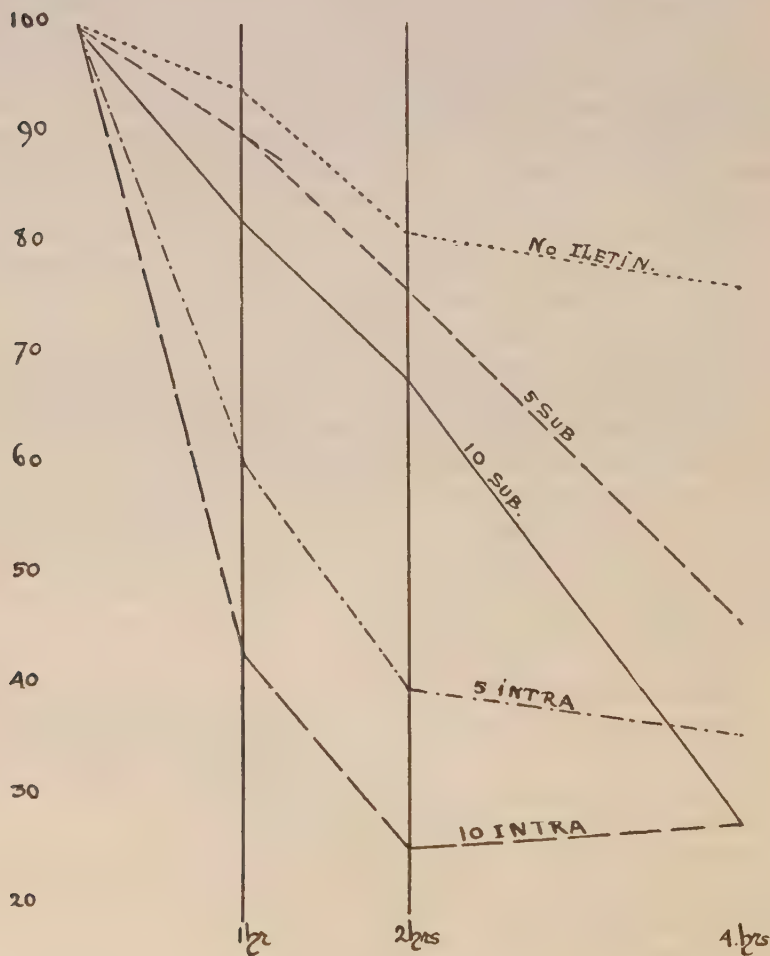


FIG. 1.

Blood sugar in per cent of initial value, following insulin injection.
Diabetes Mellitus (moderately severe).

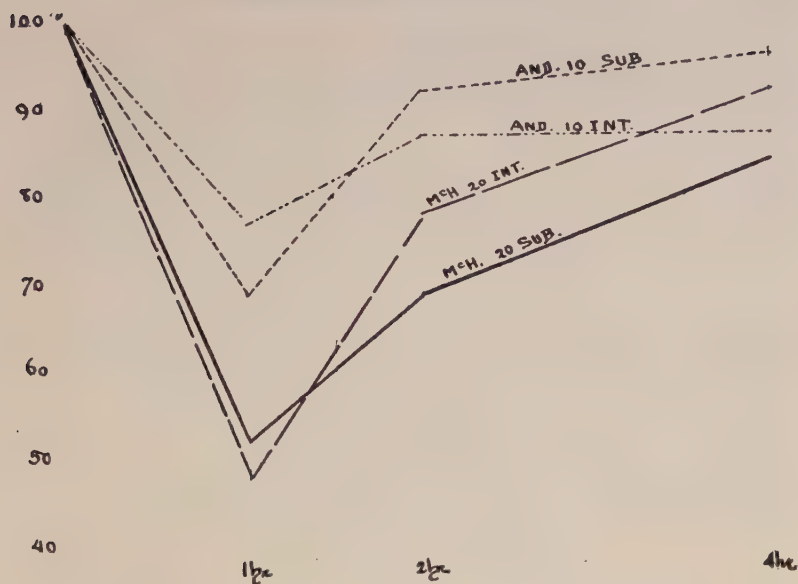


FIG. 2.

Blood sugar in per cent of initial value, following insulin injection in non-diabetics.

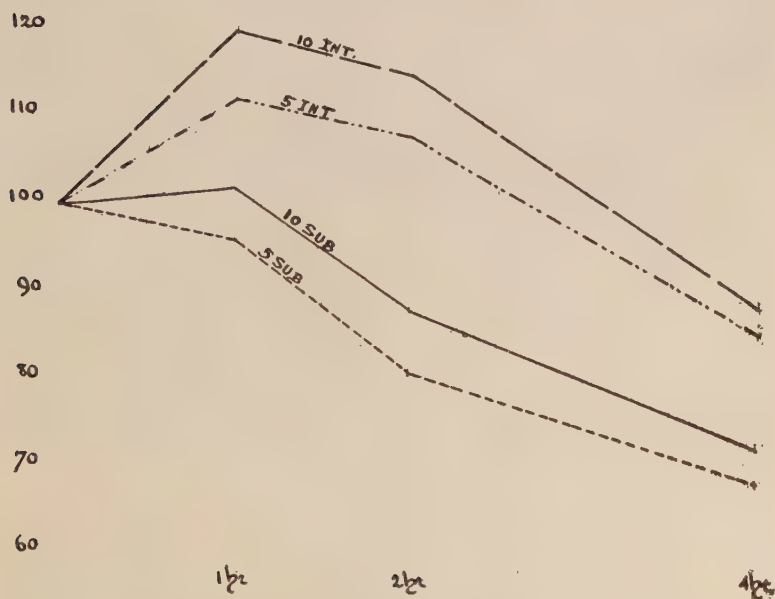


FIG. 3.

Severe diabetes mellitus.

series. The average of the absolute values in mg. per cent of the sugar destroyed when the blood was allowed to remain in the incubator, at 37° C., for two hours were as follows:

	Insulin by intradermal method	Insulin by subdermal method
Before injection	24 mg. per cent	21 mg. per cent
One hour after injection.....	19 mg. per cent	28 mg. per cent
Two hours after injection.....	22 mg. per cent	30 mg. per cent
Four hours after injection.....	15 mg. per cent	21 mg. per cent

As may be seen from the table the one-hour post intradermal injection blood failed to manifest greater glycolytic activity than did the blood drawn before insulin injection. On the other hand the one hour post subcutaneous injection blood manifested a greater glycolytic activity than did the blood drawn before the injection. In fact the glycolytic activity of the bloods drawn following intradermal injection remained constant or diminished slightly as the blood sugar level decreased, whereas by the subcutaneous method the glycolytic activity of these parallel bloods was increased at the two-hour post injection period as well as at the one-hour post injection period.

In severe diabetes the glycogenetic function of the liver is markedly reduced or is even absent. The severity of the diabetic condition is mirrored by the results of the various series as done in these experiments in that the concentration of glycogen in the liver cells governs the degree of difference in response to the two methods of injection.

This is well illustrated by Figure III from a case with severe diabetes mellitus.

Experiments done by one of us⁵ on rabbits proved that if the parasympathetic pathways are blocked by atropin the normal blood sugar lowering effect of intradermally injected insulin does not appear. In control tests it was shown that atropin itself in the doses used does not effect the blood sugar level. If rabbits are injected with atropin every 30 minutes, and the parasympathetic pathways are thus cut off during the entire period of observation, the result upon the animal's reaction to various methods of insulin administration is as follows: The blood sugar curve following intravenous administration is the same as the curve in the same animal without atropin. After subdermal injection the hypoglycemic effect is slightly less marked than in the

⁵ E. F. Mueller. To be published.

non-atropinized animal. If the insulin is administered intracutaneously in an atropinized rabbit the blood sugar level is not affected for a period of almost an hour. During the two succeeding hours a hypoglucemic effect is noted, but it is only half as great as in a non-atropinized animal.

These experiments indicate that the fibres of the parasympathetic nervous system may carry the specific stimulus initiated by the insulin injection. That area in the body which is most rich in the parasympathetic nerve endings will serve as the most effective area for the localization of the depot if the reaction to this effect is to be given the greatest prominence. This effect is totally lacking in the intravenous administration, partly demonstrable by the subdermal administration, and very much more definitely present when the insulin is deposited intracutaneously. The degree of intensity of this stimulus does not depend upon the dosage; the effect of this stimulus, though gradually diminishing, will persist as long as the deposit of active insulin remains. With the beginning of resorption of the insulin from the depot, wherever located, into the circulation the hormone effect is initiated and

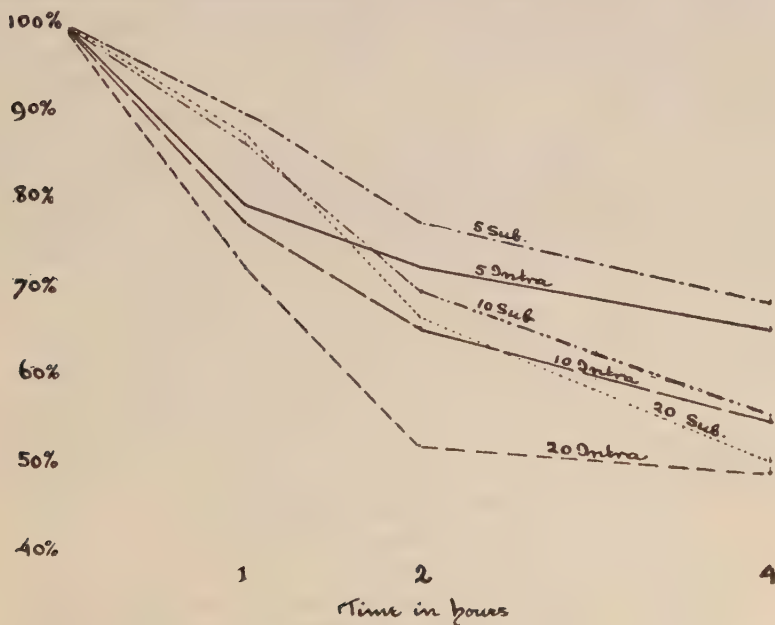


FIG. 4.

Blood sugar in per cent of initial value, following insulin injection.
Composite curves.

increases with increasing concentration of the active insulin in the circulation. As resorption into the circulation proceeds, the hormone effect increases and reaches its maximum, and the specific effect of the stimulus via the parasympathetic nervous system gradually diminishes and disappears.

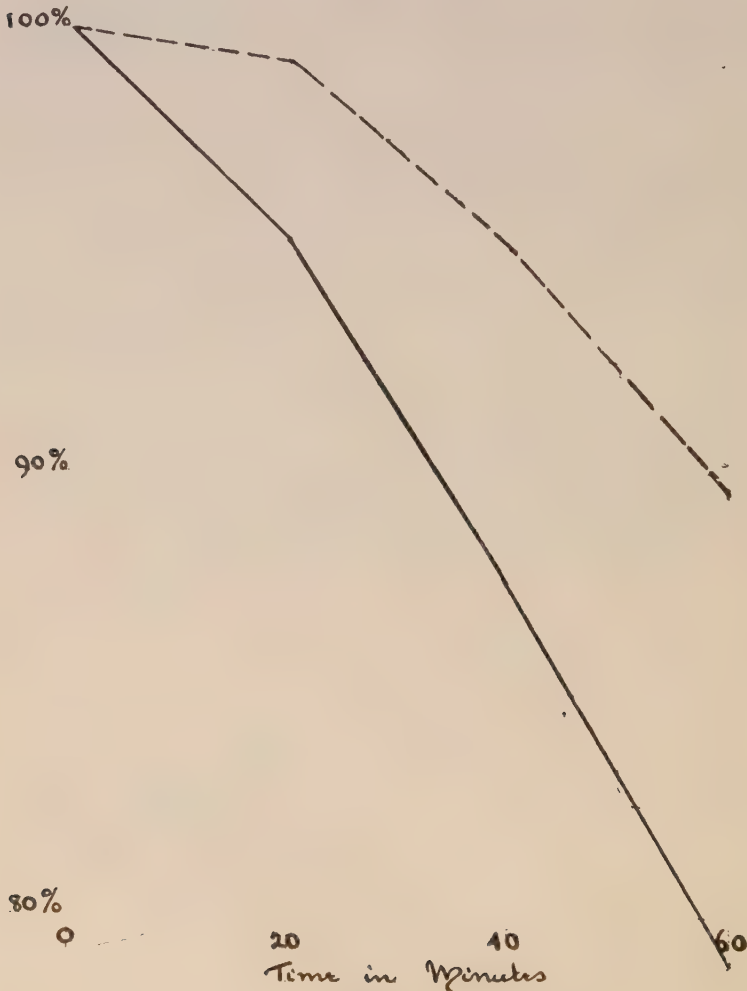


FIG. 5.
Blood sugar in per cent of initial value, following insulin injection.

———— Intradermal.
----- Subcutaneous.
Composite curves.

181 (2704)

A physiological study of the development of the collateral circulation in the leg of the dog.

By HENRY W. FERRIS and SAMUEL C. HARVEY.

[*From the Departments of Surgery and Physiology, Yale University School of Medicine, New Haven, Conn.*]

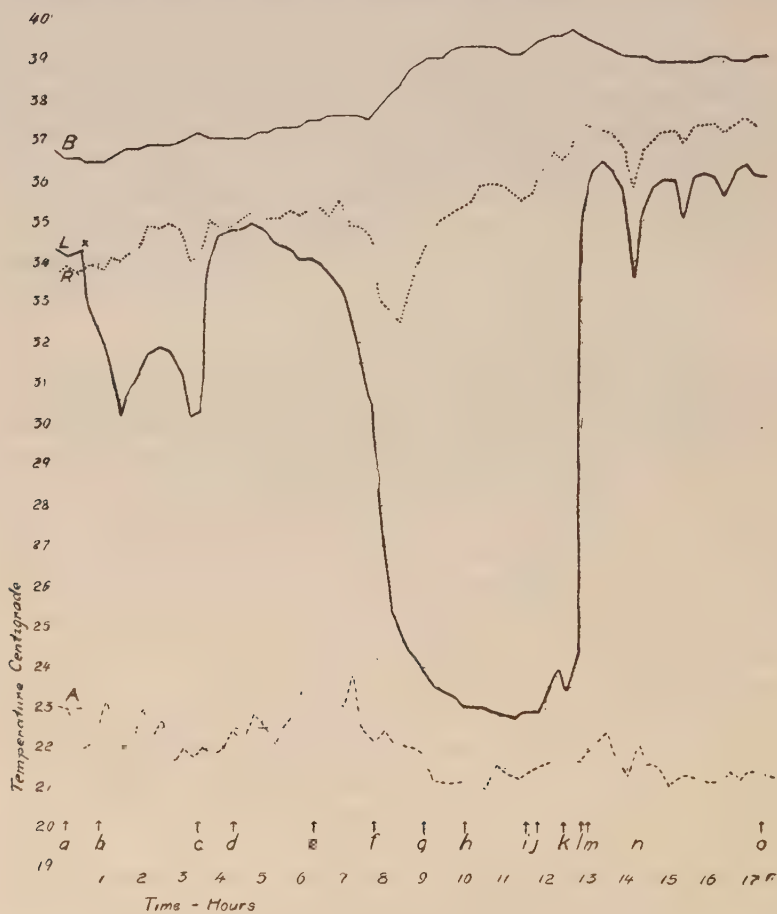
It has been known for many years that on ligating the main vessel in the leg of an animal, the temperature in that limb will fall, and that at some later time it may return to normal with the development of a collateral circulation. In reviewing the literature, however, we have been unable to discover any work which would show just how soon such a response takes place, and in just what manner. Consequently, we undertook the following experiments.

As a means of determining the flow of the blood in the limb, and the efficiency of the circulation, continuous record was kept of the temperature of the foot after tying off the superficial femoral artery on the one side, using the opposite limb as a control. This was done by means of two thermo-couple junctions soldered within needles and pushed into the foot pads of the two hind legs, according to the method of Barney Brooks.¹ For these junctions, German silver and copper wires were used. The copper wire from each junction was connected with one pole of a two-way switch. The German silver wire from each needle was joined to another piece of copper wire to form the "cold" junction, and the two were passed through a glass tube in the cork of a thermos bottle. From this cold junction in each case connection was made by the copper wire to the corresponding pole of the switch. By throwing the switch to the right or left, the thermo-electric current from one or the other needle could be sent into a galvanometer whose deflections were first calibrated according to known variations of temperature in order to transform the results into temperature readings. As an anesthetic we used amytal (iso-amyl ethyl barbituric acid), given in solu-

¹ Brookes, Barney, and Jostes, Fred A., A Clinical Study of Diseases of the Circulation of the Extremities: A Description of a New Method of Examination. *Arch. of Surgery*, Nov., 1924, pg. 485.

tion intraperitoneally, 0.055 grams per kilo of body weight of the dog.

The course of the experiment in dog No. 6 will be given as an example of the results in the series: The animal, a male, weight 20.5 kilograms, was given the calculated dose of 0.055 gm. of amytal per kilo, but it was necessary to repeat this one hour and ten minutes later to secure sufficient anesthesia. As soon as the



Dog VI. Male. Weight: 20.5 Kg.

A. Room temperature.

B. Rectal temperature of dog.

R. Temperature of right hind leg.

L. Temperature of left hind leg.

X. Artery ligated.

dog became unconscious, his body was wrapped in bagging, and an incision made in the left leg down to the femoral vessels. Two ligatures were placed around the artery in the upper part of Hunter's Canal but not tied. These ligatures were just distal to the saphenous artery which in the dog is a fair sized vessel running down the antero-medial aspect of the leg. The needles containing the junctions were sterilized in alcohol and inserted a distance of about one half inch, one into the central foot pad of each of the hind feet. A mercury thermometer was placed in the rectum, and a second to register room temperature was hung by. Readings were now taken of the temperature in both hind legs, the rectal and the room temperatures. When the temperature of the limbs seemed fairly constant, the left femoral artery was doubly tied. Temperature readings were then made every fifteen minutes. A few minutes after the ligatures were tied, the skin wound was closed with a few silk sutures. The details and remainder of the experiment are best understood by consulting the accompanying graph and notes.

As may be seen the temperature of the left leg after tying the artery fell rather abruptly not quite four degrees, then rose about one and a half degrees, fell again, then rose suddenly to above the normal level. This warmth was not maintained, however, and the foot gradually cooled down until eleven hours after ligation, at which time it had fallen about twelve degrees. The temperature then rose gradually for about one and one fourth hours, when suddenly there was a rapid change, and a rise of ten and a half degrees took place in fifteen minutes. The temperature then rose less abruptly two degrees more. The rise was accompanied by a simultaneous but less rapid increase of temperature in the normal leg. Subsequent slight simultaneous fluctuations in temperature were observed in both legs for a period of four hours more, when the experiment was terminated.

At this time the left foot was quite warm to the touch, except for the toes. Ten and one half hours later the dog was found to be awake but still very drowsy, with much staggering on attempting to walk. The left foot was superficially still slightly cold to the touch. Five days later both the legs seemed of the same temperature. Twenty days after ligation, the dog was sacrificed, and the left femoral artery found fibrosed between the two ligatures, but patent above and below.

Of a total of five dogs, on whom similar observations have been made, four have shown similar sudden rise of temperature, while the fifth apparently had a very efficient collateral circulation already in existence, so that there was only a small drop in temperature after tying the vessel.

From the rapidity of the rise in temperature it must be concluded that the first step in the establishment of a collateral circulation is a surprisingly abrupt vasomotor reaction. Further work is being carried out in an effort to analyze and determine the exact mechanism of this reaction.

182 (2705)

The effect of immersing and tearing *Amoebae* in salt solutions.

By PAUL REZNIKOFF and ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

In a previous publication¹ the effect of the injection of salts into *Amoebæ* was described by us. The results of those experiments afford criteria for judging the action of the substances directly on the protoplasm within the cell. From those studies a fuller conception may be obtained of the permeability to the salts when *Amoebæ* are immersed in solutions, and also some idea of surface reformation when the *Amoebæ* are torn in the same salt solutions.

Immersion of *Amoebæ* in different salt solutions shows that in order of increasing toxicity the salts range as follows: MgCl_2 , CaCl_2 , NaCl , KCl . Thus *Amoebæ* will live for 24 hours in M/6.5 MgCl_2 ; for about 5 hours in M/6.5 CaCl_2 ; for less than 1 hour in M/6.5 NaCl ; and for less than 1 hour in as dilute a solution of KCl as M/104. *Amoebæ* will live for three days or more in M/13 MgCl_2 , M/26 CaCl_2 , M/52 NaCl and M/832 KCl . The *Amoebæ* which die in NaCl and KCl show a sinking and clumping of the

¹ Chambers, R., and Reznikoff, P., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, xxii, 320.

granules, and resemble in this respect the *Amœbæ* into which the salts have been introduced by injection. Another interesting feature of the immersion experiments is that the *Amœbæ* dead in NaCl have a loss in the integrity of the pellicle. This does not occur with the other salts.

Comparing these results with those obtained by injection, it is evident that NaCl and KCl are much more toxic to *Amœbæ* when in contact with their external surface than when injected. With CaCl_2 and MgCl_2 the reverse is true (injection toxicity: MgCl_2 and $\text{CaCl}_2 > \text{NaCl}$ and KCl). Furthermore from the appearance of the dying *Amœbæ* there is evidence that Na and K can penetrate fairly easily, while Ca and Mg cannot.

If *Amœbæ* are immersed in mixtures of NaCl and CaCl_2 , or of KCl and CaCl_2 , the toxic effects of the NaCl and KCl are antagonized by the CaCl_2 . There is a considerable range in the lethal doses of NaCl and KCl when mixed with CaCl_2 . This variation decreases as the strength of the solutions of NaCl and KCl increases. The amount of CaCl_2 necessary to antagonize the toxic action of NaCl and KCl is as follows:

Strength of NaCl	No. of Mols. of NaCl antagonized by 1 Mol. of CaCl_2		
	Min.	Max.	Mean
M/13	60	80	70
M/26	120	160	140
M/52	180	240	210
M/104	380	540	460

Strength of KCl	No. of Mols. of KCl antagonized by 1 Mol. of CaCl_2		
	Min.	Max.	Mean
M/26	40	60	50
M/52	60	80	70
M/104	60	80	70
M/208	80	160	120
M/416	80	160	460

From the injection and immersion experiments it was seen that the effect of Na and K upon the protoplasm was to cause a sinking of the heavier granules. These granules tend to clump

at the lower surface of the Amœbæ, and persist in this condition until the Amœbæ die or recover. Although CaCl_2 is able to antagonize the lethal effect of NaCl and KCl , nevertheless some clumping occurs. Since we believe the penetrating power of Ca into Amœbæ is negligible, it is suggested that the antagonism of Ca to the lethal action of Na or K occurs on the surface of the Amœbæ.

Amœbæ torn in water rapidly repair their surfaces after the outflow of some of their contents. This debris is soon pinched off. When Amœbæ are torn even slightly in solutions of NaCl in concentrations of $\text{M}/13$ and stronger, no repair occurs. The contents pour out, the pellicle disintegrates, and the Amœbæ is rapidly dissipated. With solutions weaker than $\text{M}/13$ repair from tears takes place with increasing ease as the concentration diminishes. In a $\text{M}/104$ solution the Amœba can recover from a very extensive tear as easily as in water. In $\text{M}/13$ KCl the Amœbæ cannot recover from the slightest tears, and can recover from extensive tears only in a $\text{M}/312$ solution. In $\text{M}/6.5$ CaCl_2 there is no repair after even the slightest tears. The part of the Amœbæ near the tear sets, and this setting proceeds into the Amœbæ, the living part of which attempts to pinch off the coagulated portion. The Amœba is thus converted into a gradually lengthening column of coagulated material with a part still living at one end. This, in its turn, finally succumbs. The solidified column shows a series of constrictions which indicate the unsuccessful attempts of the Amœba to pinch off. As the strength of CaCl_2 is decreased, the Amœba succeeds in pinching off the solidified region; and in a $\text{M}/104$ solution quite extensive tears can be repaired. MgCl_2 is the most toxic of all the salts which are torn in the solutions. No repair occurs in a $\text{M}/52$ solution from even a very slight tear, and recovery from extensive tears cannot take place until the solutions are diluted to $\text{M}/832$.

The antagonistic action of CaCl_2 to $\text{M}/13$ NaCl with respect to the repair of the torn surface is most efficient in dilutions of CaCl_2 , $\text{M}/52$ to $\text{M}/208$. Solutions of CaCl_2 outside this range are not effective. CaCl_2 antagonizes the action of $\text{M}/13$ KCl in its prevention of repair of the torn surface only in dilutions of $\text{M}/208$ to $\text{M}/416$.

CONCLUSIONS.

(1) These experiments emphasize the liquefying action of NaCl and the solidifying effect of CaCl_2 brought out previously.¹

(2) They also indicate that Na and K can penetrate the Amoeba much more effectively than Ca or Mg.

(3) Of the four salts studied, only NaCl has a disintegrative action upon the surface.

(4) The tearing experiments indicate that Ca and Mg, which have very little effect on the intact Amoeba, enter the cell easily through a tear. The action of Ca is localized, and the Amoeba rids itself of the dead portion by an active "pinching-off" process. Mg, on the other hand, tends to diffuse throughout the cell, and is therefore extremely toxic. These findings substantiate the results obtained in the injection experiments.

(5) To antagonize toxic effects of NaCl or KCl, CaCl_2 must be present in a non-lethal dose.

183 (2706)

The sex ratio in litters of mice classified by the total amount of prenatal mortality.

By E. CARLETON MacDOWELL and ELIZABETH M. LORD.

[*From the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N. Y.*]

From studies of the normal sex ratio of living young and of abortions and still-births in man and the rat, King¹ and others have concluded that the male foetus is less viable than the female. If this is true for the entire period of gestation there should be a negative correlation between the amount of prenatal mortality and the sex ratio. From counts of the corpora lutea of pregnancy of about 20 mice (sectioned material) Parkes² concludes that this is the case.

¹ King, H. D., *Anat. Record*, 1921, xx, 321.

² Parkes, A. S., *Proc. Roy. Soc.*, 1923, xcv, 551.

Data showing the percent of prenatal mortality for 445 litters of mice have been obtained by subtracting the number of young born from the number of ova as indicated by the number of corpora lutea. In the last week of pregnancy the corpora lutea corresponding to the litter *in utero* are strikingly differentiated as large hyperemic bodies protruding from the surface of the otherwise pale colored ovary. These corpora were counted in the living animals under a low power binocular microscope by means of an operation which has been shown³ to have no influence upon the foetuses *in utero* or upon the subsequent reproduction. Table I gives the total number of males and females in litters classified, according to the per cent of prenatal mortality, into five classes, each 20 per cent in width. The sex ratio shows no tendency to decline as the percentage of prenatal mortality increases; the highest ratio is found in the next to highest prenatal mortality class. The small numbers in the fifth class indicate that its low sex ratio is probably not significant. These results indicate that when the total prenatal mortality is revealed there is found no selective elimination of males.

TABLE I.

% prenatal mortality	0-19.9		20-39.9		40-59.9		60-79.9		80-99.9	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of mice born	497	483	362	362	269	262	120	116	21	33
Males per 100 females	102.9		100.0		102.7		103.4		63.6	

³ MacDowell, E. C., *Anat. Record*, 1924, 329.

184 (2707)

The flocculation of botulinus toxin antitoxin mixtures.

By J. BRONFENBRENNER and P. REICHERT.

[From the Laboratories of the Rockefeller Institute for Medical Research, New York City.]

Neutral or nearly neutral mixtures of diphtheria toxin and antitoxin give rise to a flocculent precipitate, appearing earliest in those tubes approaching most nearly the point of toxin neutralization. This phenomenon was adapted by Ramon¹ to the *in vitro* titration of antitoxin, a method the value of which has since been confirmed by a number of investigators.

In the course of an attempt to develop an early specific reaction for the diagnosis of botulism it was hoped that the precipitin test might be useful to detect small quantities of toxin in the circulating blood of laboratory animals suffering from this disease. For the production of the precipitating serum, rabbits were immunized with the formalinized filtrates of four day old botulinus cultures. Before the addition of formalin these filtrates contained approximately 100,000 guinea pig MLD per cc.

The sera obtained were titrated in mice, and 0.1 cc. was found to protect against 6,000 fatal doses. These sera, however, were not flocculated by their homologous toxin. Toxins of various age and potency, and at various pH all gave negative results. A sample of botulinus antitoxin received from the New York City Department of Health gave only a weak and irregular reaction; 0.1 cc. of this serum protected a mouse against 10,000 fatal doses. Under parallel conditions the diphtheria toxin antitoxin mixtures gave typical quantitative precipitations.

We had previously observed that precipitation takes place when the antitoxin is combined with the extracts of foods infected with *B. botulinus*,² as well as with bacterial autolysates, although these reactions were found to be not strictly type speci-

¹ Ramon, G., *Compt. rend. de la Soc. de Biol.*, 1922, lxxxvi, 711; *Ann. de l'Institut Pasteur*, 1923, xxxvii, 1001.

² Bronfenbrenner, J., and Schlesinger, M. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1919, xvii, 24, 25.

fic.³ In the light of these observations the negative results obtained above led us to suspect that the appearance of the precipitate might be due, not to the actual antitoxin content, but to the presence in the serum of an antibacterial antibody. A new set of rabbits was accordingly immunized with the filtrates of 16-24 day old cultures of *B. botulinus* similarly treated with formalin. These older filtrates were actually somewhat less toxic than the four day filtrates, yet the antitoxic sera obtained were flocculated sharply by the original antigen and by other old filtrates. The four day filtrates used above flocculated these sera definitely but more weakly and in a narrower zone.

This is illustrated by the following composite protocol:

Amount of Antigen used in cc.	0.05 cc. of Antiserum to a 4-day toxin		0.05 cc. of Antiserum to a 16-day toxin	
	Flocculated by a 4-day toxin	Flocculated by a 16-24-d. toxin	Flocculated by a 4-day toxin	Flocculated by a 16-24-d. toxin
1.000	—	—	—	—
.750	—	—	—	—
.500	—	—	—	—
.300	—	—	—	—
.200	—	—	—	—
.150	—	—	—	weak
.100	—	—	—	+
.075	—	—	—	+
.050	—	—	—	+
.030	—	—	weak	+++
.020	—	—	+	+
.015	—	—	weak	weak
.010	—	—	weak	weak

It is evident that a young toxin, though it be physiologically more potent than an older one and productive in animals of a highly antitoxic serum, may not produce precipitins, whereas a filtrate from a culture old enough to contain presumably a relatively high concentration of bacterial protein in addition to toxin produces a flocculating as well as antitoxic serum when used for immunization of animals.

The use of flocculation for the *in vitro* titration of botulinus antitoxin is now shown, therefore, to be limited by the fact that the flocculating power is not strictly parallel to toxicity but depends upon the presence of bacterial proteins in the antigen. That

³ Bronfenbrenner, J., Schlesinger, M. J., and Calazans, J. C., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 21.

a botulinus toxin rendered nontoxic—by formalinization, for instance—still retains its flocculating power constitutes a further restriction. In the case of the titration of diphtheria antitoxins this latter restriction has already been definitely pointed out.^{4, 5}

185 (2708)

Permanence of results obtained by *L. acidophilus* therapy.

By NICHOLAS KOPELOFF.

[From the Department of Bacteriology, Psychiatric Institute,
Ward's Island, New York City.]

Having demonstrated that constipation and diarrhea are alleviated during the ingestion of *L. acidophilus* milk, it is of interest to establish whether such benefits are permanent or transitory. In other words, is it necessary to continue the ingestion of *L. acidophilus* milk in order to maintain the benefits of treatment?

Of 34 cases where daily observations have been carefully recorded after discontinuing treatment, 27 cases, or 80 per cent continued to have more normal defecations; 6 cases, or 18 per cent, had approximately the same number, and 1 case, or 3 per cent, had fewer normal defecations than before treatment. One case which prior to treatment averaged one normal defecation per week, had daily normal defecations *without exception* for 924 days after treatment was discontinued. Three other cases showed benefits over a year after treatment was discontinued, and 10 cases showed benefits from 3 to 6 months afterwards. There were 14 cases where the treatment was of relatively short duration, *i. e.*, about 2 months or less, and the benefits persisted for considerably longer than that time.

While the results of *L. acidophilus* therapy obviously vary from individual to individual, there is here good evidence for believing that in most instances the beneficial effects of *L. acidophilus* persist after treatment has been discontinued.¹

⁴ Glenny, A. T., and Okell, C. C., *J. Path. and Bact.*, 1924, xxvii, No. 2, 187.

⁵ Bayne-Jones, S., *J. Immunol.*, 1924, ix, 481.

¹ Kopeloff, N., *Lactobacillus acidophilus*, 1925, Williams and Wilkins Co. (In press).

186 (2709)

Metabolism of some heterocyclic compounds.

By N. JEAN NOVELLO and CARL P. SHERWIN.

[*From the Chemical Research Laboratory, Fordham University, New York City.*]

Imidazol was fed to rabbits and found to be partly exidized, thereby increasing the output of uric acid. There was no increase in sulphate or glycuronic acid output. About one-third of the substance was excreted the same as fed.

Pyridine was fed to rabbits and dogs. The methyl derivative was isolated from the dog urine, but no conjugated product found in the urine of rabbits. The nitrogen and sulphur partition was studied after the work on rabbits.

Pyrrole was fed and injected into rabbits and dogs. The nitrogen and sulphur partition was studied, but no definite detoxication isolated from the urine of rabbits.

Picric acid was fed to rabbits, and picramic acid isolated from the urine.

The relation of nitro indols to acetyl picramic acid was studied.

187 (2710)

Studies on adrenal insufficiency.

By G. N. STEWART and J. M. ROGOFF.

[*From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, Cleveland, Ohio.*]

Duration of survival after adrenalectomy.—The adrenals were removed in 36 dogs, an interval being left between the removal of the right and left. In 7 of these animals intravenous injections of Ringer's solution with dextrose added were given, usually once in the 24 hours.

Of the 29 animals which received no injection, one suffered from respiratory difficulties from the time of the second opera-

tion till its death after $3\frac{1}{2}$ days. The lungs were found congested. One dog died on the fourth day, a stitch abscess breaking into the peritoneal cavity. One dog died 26 hours after the second operation with extensive consolidation and oedema of the lungs. The remaining 26 dogs showed at autopsy no pathological changes sufficient to account for death, apart from those which are described below as associated with adrenal insufficiency. One of these animals survived the second operation for 15 days; another (in advanced pregnancy) died on the 16th day. Two died on the 10th day; 2 lived 8 days; 4 lived 7 days; 5 lived 6 days; 2 lived 5 days; 6 lived 4 days. Two died on the third day, and one lived 2 days. As our results are so different from those emphasized in the literature, it must be stated distinctly that the removal of the adrenals was complete in all our dogs as verified both at operation and *post mortem*. No accessory adrenals were found by careful macroscopic search in any of our dogs.

Of the 7 injected dogs, one is alive on the 28th day after removal of the second adrenal (having given birth to 6 pups on the 16th day, two of which are alive and being excellently nursed by the mother).¹ One of the injected dogs died on the 34th day; two died on the 21st day; one on the 16th day. The remaining two animals were shown at autopsy to be suffering from pulmonary complications; one of them died 12 days after removal of the second adrenal with oedema and congestion of the lungs; the other died on the 6th day with severe pulmonary congestion and a subcutaneous hematoma at the site of the last operation, probably due to slipping of a ligature.

In general the injections were begun the day after the removal of the second adrenal and before symptoms of adrenal insufficiency had appeared. When the injection was not begun until the animal was moribund, it was sometimes of great temporary benefit but did not seem to prolong life nearly to the same extent.

Symptoms.—The most characteristic are those pointing to a profound disorganization of the digestive mechanism. The animal usually recovers completely from the second operation in a few hours or even less. Then follows a period of apparently normal health. The appetite is good, the individual peculiarities

¹ She died near the end of the 33rd day after removal of the second adrenal. The pups are in excellent condition more than 3 weeks after birth.

in the behavior of the animal are unchanged. This period of good health is longer or shorter according to the length of time the animal survives, no notable change occurring, as a rule, until a relatively short time before death. The great symptom which announces a serious change is the refusal of food. The anorexia is usually total, and comes on rather abruptly, often accompanied or followed, occasionally preceded, by vomiting and diarrhea. Sooner or later in most cases bile appears in the vomit, and the stools are not infrequently heavily charged with bile. Blood may be seen sometimes in the vomit or the stools or in both. Once the typical anorexia has appeared it is practically always permanent till death with the exception that the animal may be tempted once or twice by some delicacy. The picture is essentially that of a severe and permanent gastro-intestinal upset. In this the symptomatology agrees with the pathological changes found *post mortem*.

Weakness is not necessarily a prominent symptom until near the end. It is especially evident in the hind legs when the animal tries to walk or stand. The legs are widely separated so as to widen the base of support, they wobble, move stiffly and the movements are poorly coordinated. Convulsions, with or without coma, and apparent hallucinations accompanied by a peculiar cry, are occasionally observed. Exaggerated reflex excitability, with twitching of the limbs, etc., is more common.

Symptoms of circulatory disturbance (slow and often irregular pulse, but sometimes a very rapid pulse) are frequently found shortly before death. The rectal temperature may be subnormal, but is often normal up to the time of death.

In the animals treated by injections, the same general symptoms ultimately come on, but their onset is postponed, roughly speaking, in proportion to the lengthening of the survival period. In not a few instances it has been observed that some of these symptoms, even when quite pronounced (including the asthenia, the nervous, circulatory and perhaps less often the digestive symptoms), disappeared under the treatment, and that more than once in the same animal.

Autopsy findings.—Almost in every case more or less blood or blood pigment is found in the contents of one or more portions of the gastro-intestinal tract. Not infrequently the hemorrhage is considerable. Blood is not seldom found in smaller or larger

amount during life in the feces or vomit. It is not found in the esophagus unless the animal has recently vomited. Bile is generally abundantly present in the alimentary canal, usually in the stomach as well as in the intestine. The bile in the gall bladder does not contain blood or blood pigment when the autopsy is performed immediately after death, as was the case in the great majority of our animals.

Marked congestion and extensive hemorrhages are frequently encountered *post mortem* in the mucosa of one part or another of the gastro-intestinal tract (stomach, pyloric end of duodenum, small intestine, especially the upper end, and colon, never in the esophagus). Some congestion is the rule. Marked congestion of the pancreas is frequent.

The beneficial effect of the injections is of course compatible with the idea that in the absence of the adrenals toxic substances accumulate which are responsible for the pathological changes and the symptoms. If these are being eliminated by way of the gastro-intestinal mucosa, it is not difficult to see that the injection of solutions which are known to be in part excreted into the intestine, at least in the first instance, might aid in washing out the toxic substances and in diluting them, so that the concentration necessary to produce symptoms and to cause damage to the gastro-intestinal mucosa or other tissues would not be so easily reached. This is not the only way in which such injections might act. The one thing clear is that they cannot supply the missing cortical hormone if there is one, the absence of which might be specially associated with the disorganization of the digestive mechanism.

188 (2711)

Reaction to Gram's stain by certain spore-forming bacteria.

By GEORGE G. DE BORD.

[From the Dairy Department, Iowa State College, Ames, Iowa.]

It has been customary to consider that the greater number of the spore-forming bacteria are positive in their reaction to Gram's stain. The positive correlation of these two properties has been

considered very nearly perfect with the mesophilic bacteria. It might be expected that the same correlation would be obtained with the thermophilic bacteria. Various investigators working with the spore-forming thermophiles have reported some of these organisms to be Gram negative. The stain was made usually upon cultures at the end of 24 hours', and in some cases 48 hours', incubation. These statements would give further exceptions to the Gram-positive/spore-formation correlation of the bacteria, when their temperature requirements are disregarded.

It has been the experience of the author that some of the spore-forming thermophilic bacteria are Gram negative at the end of 24 hours' incubation. These organisms, however, were positive in younger cultures. Many strains began to lose their power of retaining the violet stain at the end of 8 hours' incubation. Opinion might differ as to whether this would destroy the Gram-positive/spore-formation correlation. The fact, however, that the organisms were positive in young cultures should be sufficient evidence to prove that the correlation is confirmed. It is probable that the change in the staining reaction has a metabolic significance, which might be utilized in systematic bacteriology. It is believed that this characteristic should be investigated and the results recorded with the description of the organism.

189 (2712)

Rôle of the intestinal blood vessels in canine anaphylaxis.

By W. H. MANWARING, F. I. O'NEILL and MARGERY McCULLOUGH.

[*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*]

The stomach, intestines and spleen of dogs were removed without interfering with the hepatic circulation. This was done by connecting the portal vein with the abdominal *vena cava* by means of a transfusion cannula, the return circulation from the hind quarters replacing the normal portal circulation. Dogs thus partially eviscerated give typical anaphylactic reactions on intravenous injection with specific foreign protein, the arterial

blood pressure falling to a third, even to a quarter, of the normal arterial pressure within two minutes.

The intestine, therefore, is not the dominant site of the extra-hepatic vascular reactions in canine anaphylaxis, as currently assumed.

190 (2713)

The action of glycerol on the virus of experimental typhus fever and on *Proteus* bacilli.

By PETER K. OLITSKY

[From the Laboratories of the Rockefeller Institute for Medical Research, New York City.]

Glycerol was employed as a preliminary to the study of an agent capable of destroying the infective but not the immunizing property of the virus of experimental typhus fever in the guinea pig.

Fragments of brain removed from typhus-infected guinea pigs on the second day of the fever were immersed in 1, 5, 10, 25 and 50 per cent sterile glycerol, and placed at a temperature of about 6° C. After a period varying from 7 to 22 days in the case of the lower dilutions, and from 7 to 58 days in the case of the 50 per cent dilution, approximately 2,000 minimal infecting doses of the virus contained in the washed glycerolated brain tissue were injected intraperitoneally into normal guinea pigs. The results in all cases showed that the brain was deprived not only of its infective but also of its immunizing action.

The etiological significance in typhus fever, lately ascribed to *Bacillus proteus* X₁₉ by several investigators, suggested a test of the influence of glycerol on this micro-organism. Accordingly, 24 hour agar slant cultures of *Bacillus proteus* X₂ and X₁₉ were washed off with the same dilutions of glycerol as had been used in the case of the typhus virus, and the glycerolated cultures were kept in the ice-box. At weekly intervals sub-plants were made on agar plates of 0.2 cc. of the glycerol suspensions. After 3½ months an infinite number of colonies was noted in the case of both strains, and in all dilutions, except that of 50 per cent, from which no growth could be obtained after one month.

The resistance of the bacilli contained in the brain was tested by injecting cultures of X_2 and X_{19} strains intraperitoneally into normal guinea pigs. The animals died in 24 to 48 hours, having developed septicemia, fibrinopurulent peritonitis and hemorrhagic splenitis—a pathological picture wholly different from that induced by the pure typhus virus. The brain, which yielded cultures of *Proteus bacilli*, was cut into fragments, immersed in 1 and 50 per cent glycerol and kept in the ice-box. Interval cultures of this glycerolated brain showed profuse growths of the *Bacillus proteus* for as long as 3 weeks. After one month the material in the 1 per cent glycerol yielded a sparse growth of *Bacillus proteus*, and that in the 50 per cent glycerol gave no growth.

In conclusion, *Bacillus proteus* X_2 and X_{19} , either in culture or in the brain of *Proteus*-infected guinea pigs, is quite resistant to the action of glycerol, and differs markedly in this respect from the typhus virus. Under these experimental conditions the infective and immunizing properties of the pure virus are very susceptible to the destructive action of glycerol.

191 (2714)

The effect of the scorbutic state upon the production and maintenance of intercellular substances.

By S. BURT WOLBACH and PERCY R. HOWE.

[From the Harvard Medical School, Boston, Mass.]

Guinea pigs in the condition of absolute scorbutus, *i. e.*, complete deprivation of anti-scorbutic substances in the diet, have been studied, and the effects of the administration of anti-scorbutics noted. The diet employed was: Soy beans, 50 grams; rolled oats, 28 grams; dried skimmed milk (Klim), 10 grams; yeast, 4 grams; butter, 5 grams; calcium carbonate, 1 gram; sodium chloride, 1 gram. For roughage, the guinea pigs were liberally supplied with filter paper. Control guinea pigs, which received the same diet, with the liberal administration of orange-juice, or green vegetables, remained healthy.

The effects have been studied in growing guinea pigs and in the repair of lesions of bone experimentally made. The earliest

effect of the scorbutic state is to be found in the incisor teeth, and is evidenced by the manner in which the formation of dentine is affected and in changes in the layer of odontoblasts. The earliest changes may be observed in six to seven days, while a few days later very striking conditions are found, among the most noticeable of which is the separation of the layer of odontoblasts from the dentine. This space is presumably filled by liquid. The odontoblasts undergo striking changes in regard to size, arrangement and staining reaction. The effect of the administration of an anti-scorbutic upon this condition is apparent within forty-eight hours, and results in the prompt formation of dentine so as to fill the space caused by the separation of the layer of odontoblasts. In the bones, formation of bone ceases immediately, while osteoblasts in certain locations, particularly beneath the periosteum, continue to proliferate. This applies both to flat bones and long bones. Accumulations of osteoblasts of considerable size may occur before hemorrhages take place. That the cells under consideration are osteoblasts is proved by the effect of the administration of a single dose of an anti-scorbutic substance, because it is followed by the prompt appearance of bone matrix between the cells. A single administration of orange juice produces an effect which is easily demonstrable forty-eight hours later. In the incisions through the cortex of bone in the scorbutic state there is no bony repair, while controls operated on in the scorbutic state, but given anti-scorbutic substances after the operation, show new bone formation very promptly. The repair of soft tissues is likewise very markedly affected. Proliferation of fibroblasts is apparently but little affected by the scorbutic state, but there is a marked diminution in the amount of intercellular substance formed. There is also a very marked effect seen in the retardation of new blood vessel formation in the organization of lesions, etc.

Other observations were made on the effect of the scorbutic state upon bone previously formed and upon cartilage.

Summary and Conclusions.

The scorbutic state may be characterized as one affecting supporting tissues in which the cells are unable to produce and maintain intercellular substances. This condition affects various supporting substances to a different degree, and is most marked in those in which the intercellular substance is calcified, as the den-

tine of teeth and the matrix of bone. The characterization applies to cartilage and connective tissue, and, by inference, to other intercellular substances, including that of blood vessels. The hypothesis is entertained, based upon the study of repair in incisor teeth of scorbutic guinea pigs, that in the formation of intercellular substances there is a change of the material from a liquid to a solid or jell state, and that the missing factor in the scorbutic condition is one affecting the jelling or setting of a liquid product.

192 (2715)

The epithelial tissues in experimental Xerophthalmia.

By S. BURT WOLBACH and PERCY R. HOWE.

[*From the Harvard Medical School, Boston, Mass.*]

Standard strains of white rats obtained from the Wistar Institute were employed in this study. The diet employed was practically that of Mendel, as used by Yudkin and Lambert¹: Casein, 18 grams; mineral salts (Mendel's mixture), 4 grams; starch, 54 grams; lard, 24 grams; yeast extract (Harris), 0.1 gram. Control rats on the same diet, but with fresh butter in place of lard, have been maintained in a healthy condition since August, 1924. On the deficient diet, rats exhibited the usual external evidences of the xerophthalmic condition in from seven to sixteen weeks, according to their age. The rats which died in advanced xerophthalmia, or which were sacrificed, presented a few very striking gross pathological changes, in addition to the pathology of the eyes, which is so well known. Most conspicuous among these changes are the presence of cavities at the base of the tongue and in the pharynx filled with a yellow cheesy material; and similar abscess-like cavities in the sub-maxillary glands. In male rats, very striking changes are to be observed in the prostate glands and seminal vesicles. These organs may be shrunk, yellowish in color, and filled with opaque yellowish spots. These gross changes are readily accounted for by the microscopic examination. The cavities described are in reality cysts lined with a stratified keratinizing epithelium, and the cheesy contents is a

¹ Yudkin and Lambert, *J. Exp. Med.*, xxxviii, 1.

mixture of desquamated keratinized cells and leucocytes. A careful microscopic study of rats killed at different stages in the development of advanced xerophthalmia shows that the morphological concomitants in the condition are limited practically to epithelial structures. While interesting transitional stages may be observed, and are being studied, the condition of xerophthalmia results in the transformation of various epithelia into a stratified squamous keratinizing epithelium. This change is practically constant in the upper respiratory tract, including the whole of the nasal passages, larynx, trachea, and bronchi. In the digestive tract, the stomach and intestines have so far exhibited no change, but all the salivary glands and the accessory salivary glands are affected, and frequently show cavities resulting from the retention of desquamated keratinized epithelial cells. Similar changes occur late, in the pancreas. In the genito-urinary tract, this transformation into keratinized epithelium is found in the renal pelvis, bladder, seminal vesicles, epididymis, and prostate gland. In all of the glands noted above there is a considerable degree of atrophy before the change in character of the epithelium takes place.

Other changes observed include a striking atrophy of the thyroid gland, testes, and paraocular glands. The complete histological sequence involved in this change in structure of epithelium has not been worked out. In most instances in glands, the earliest changes are noted in the ducts. The change is an abrupt one, beginning in foci and spreading. This change is attended by the acquisition of intense basic staining properties on the part of the cells, and these cell clusters lie in the lowest layer of the mucosa and have at the first the appearance of minute independent new growths. Gradually, however, the whole of the normal mucosa becomes replaced by the new type of epithelium. Preliminary mitochondrial studies indicate that there is no intimate relationship between the maintenance of mitochondria and the transition in type of epithelium.

Conclusions:

Deprivation of the anti-xerophthalmic vitamine (fat soluble A) affects specifically epithelial tissues. This effect is manifested in cells having presumably widely different chemical (secretory) functions, and terminates in the complete loss of specific function and the transformation into a common type of chemically inactive (non-secretory) epithelium.

PEKING BRANCH

University of Peking Medical School, February 25, 1925.

193 (2716)

The acute toxicity of ephedrine.

By K. K. CHEN.

[From the Laboratory of Pharmacology of Peking Union Medical College, Peking, China.]

In our preliminary report,^{1, 2} mention was made concerning the toxicity of ephedrine in rats and rabbits. As the drug is possibly of clinical importance in the treatment of asthma and hypotension as demonstrated in the Peking Union Medical College Hospital, it is thought desirable to study its toxicity more in detail.

Ephedrine sulphate was used in all our experiments.

In frogs, the M.L.D. by injection into the anterior lymph sac varies from 530 to 680 mg. per kilo of body weight. The animal shows weakness in the legs half an hour after the injection of such a dose, and dies in 2 to 3 hours without any noticeable convulsions.

Of the mammals investigated, the white rat appears to be most tolerant to the drug. By intravenous injection, the M.L.D. in mg. per kilo of body weight in rabbits is 66-70, in dogs 70, in cats 75, and in white rats 135-140. It appears that animals weighing over a kilo have their M.L.D. approximating 70 mg. per kilo. Death is almost immediate and follows clonic convulsions. If the chest is opened when the animal is apparently dead, the heart is seen either in fibrillation or in incomplete block. The skin and the mucous membrane do not appear to be blanched. In anesthetised animals, the M.L.D. causes an immediate and permanent fall in blood pressure to 10-15 mm. Hg, accom-

¹ Chen, K. K., and Schmidt, Carl F., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 351.

² Chen, K. K., and Schmidt, Carl F., *J. Pharm. Exp. Ther.*, 1924, xxiv, 339.

panied by a tremendous decrease of intestinal and kidney volumes. The respiratory movements may persist for a short time after the blood pressure has reached the lowest level. The fall of pressure is not prevented by artificial respiration after the destruction of the brain and the cord. Death is therefore least likely due to the failure of the respiratory center. The cardiac changes are worthy of notice. Rabbit's heart perfused by Langendorff-Locke's apparatus shows auricular and ventricular fibrillation after a large dose of ephedrine, which cannot be attributed to the slight change, if any, in the coronary outflow of H-ion concentration. The action is obviously not of central origin. Electrocardiographic studies reveal very prompt alterations in the curves after the intravenous injections of a M.L.D. There is disappearance of sinus rhythm, with the occurrence of bundle-branch-block which is finally followed by ventricular fibrillation. These changes are not due to vagal effect because they are not prevented by complete atropinization.

Recovery from sublethal doses is always complete. Repeated injections of small amounts in the same animal do not raise its M.L.D, showing that a tolerance is not developed.

By different methods of administration, the M.L.D in mg. per kilo varies; thus, in rabbits, it is 590-600 per os, 340 by intramuscular injection, 320-360 by subcutaneous injection, 310-390 by intraperitoneal injection, and 66-70 by intravenous injection. Ephedrine given other than by intravenous injection does not produce convulsions until 1 to 2½ hours after, and death in 2 to 7 hours. These convulsions are not due to cortical stimulation because they are not abolished by decerebration. There is definite change in the electrocardiogram during the convulsive stage, as illustrated by prolongation of P-R interval, widening of Q-R-S waves, and the occurrence of bigeminal rhythm due to ventricular extrasystoles.

194 (2717)

The occurrence of antipneumococcus substances in the blood serum in lobar pneumonia.

By RICHARD H. P. SIA, O. H. ROBERTSON, SHU-TAI WOO and SHEO-NAN CHEER.

[From the Hospital of the Rockefeller Institute for Medical Research, and the Department of Medicine, Peking Union Medical College, Peking, China.]

With a method¹ previously described for carrying on growth-inhibition tests with the pneumococcus, it was shown that a mixture of serum and leucocytes from naturally resistant animals (cats or dogs) was capable of inhibiting the growth of pneumococci in considerable numbers, whereas a serum-leucocyte mixture from a susceptible animal (rabbit) was found to have no such action. It was possible, however, to passively confer growth-inhibitory and pneumocidal powers on serum-leucocyte mixtures of the latter animal by adding a very small quantity of a homologous antipneumococcus serum. By comparing different immune sera the degree of growth inhibition thus obtained paralleled closely their protective action for mice. In a subsequent investigation on experimental pneumococcus infection, it was found that recovery was invariably accompanied by the development in the serum of the infected animals of immune substances, which could be demonstrated by the occurrence of pneumococcus growth-inhibition when such serum was added to rabbit serum-leucocyte mixtures. On the other hand, the serum of animals dying from the experimental disease failed to show such immune changes at any time during the course of the infection. This led to the present study of the occurrence of these antipneumococcus substances in the blood serum of patients with lobar pneumonia.

The method employed was briefly as follows: Specimens of patient's serum were obtained from blood taken by vena-puncture on various days of the illness, and kept in a refrigerator until used. The several serum samples, inactivated beforehand, were tested by adding progressive dilutions of them to normal rabbit serum-leucocyte mixtures, which were in turn seeded with a constant small number of pneumococci. The strains of pneumococci used were either those isolated from the patient yielding the test serum or a homologous stock strain. All were virulent for rab-

¹ Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1924, **xxxix**, 219.

bits. The small tubes containing the test mixtures were finally sealed with paraffined corks, and attached to a specially devised apparatus which produced constant agitation during incubation.

By the use of this method it was found that in all cases studied the serum obtained at or soon after the crisis possessed the power to inhibit the growth of the pneumococci in the rabbit serum-leucocyte mixtures; the serum obtained before the crisis, on the other hand, either totally lacked this power or showed it to only a very slight degree. The titer of these antipneumococcus substances reached the highest point three to four days following the crisis, then gradually diminished. The length of time before the immune bodies totally disappeared from the serum was not determined. In one patient who recovered spontaneously from a Type 1 pneumococcus pneumonia, the titer of antipneumococcus substances seventy days after the crisis, although markedly decreased, was still demonstrable by this technique. In the cases of Type 1 pneumonia treated with antipneumococcus serum with favorable results, these immune substances were detectable in the patient's serum soon after the intravenous therapy, and with the suspension of the treatment they tended to diminish rapidly, so that by the end of a week immune substances were usually no longer demonstrable in the patient's serum. In one patient with Type 1 pneumococcus pneumonia, however, where the first dose of immune serum was given as early as thirty hours after the onset of the disease, the titer of the anti-pneumococcus substances rose rapidly following the intravenous injections, and diminished very gradually. Thirty-three days after the last injection his serum still gave a fairly high titer of these substances, suggesting the presence of active immunity in this case. With cases that terminated fatally, on the other hand, no such antipneumococcus substances were detectable in the serum at any time during the course of the disease. This included a case of Type 1 pneumonia who died in spite of having received a total of 175 cc. of Type 1 immune serum on the fifth day. His blood serum even immediately after 100 cc. of immune serum failed to show the presence of anti-pneumococcus substances.

These findings add further support to the view that the crisis and recovery from pneumococcus lobar pneumonia are brought about by the development of specific antipneumococcus immune bodies in the blood serum, which, in conjunction with the leucocytes and normal serum constituents, bring about inhibition of growth and final destruction of the invading pneumococci.

195 (2718)

Chemical studies on the urine of eunuchs.

By T. C. SHEN. (Introduced by J. F. Kessel).

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

Read¹ and McNeal² reported the presence of creatinuria in eunuchs. The purpose of the present investigation was to make a thorough urinary analysis based upon their findings. The results, however, are somewhat different from theirs.

The eunuchs studied were selected from those recently discharged from the Manchu household. A series of four has so far been collected for careful study. No change was made in their usual diet.

They were all middle aged, ranging from 44 to 57 years, and had been castrated at ages of from 16 to 26; *i. e.*, about thirty years prior to this investigation. During the period spent in the Imperial Palace they led sedentary lives, and were accustomed to rather rich diet. But after their discharge they were almost all vegetarians. The daily food of those in my series consisted largely of millet and, to a less extent, of wheat flour and also salted vegetables; only one of them (W. C. H.) took meat in moderate quantities.

After a thorough preliminary physical examination they were kept under observation for 10 to 40 days for daily collection of urine throughout the period. They were all physically sound except one (T. W. C.) who had mild chronic emphysema and bronchitis. Repeated determinations by Van Slyke's method³ for creatin and creatinin failed to show the presence of the former in the urine, except on a few occasions when the amounts found were very well within the limit of analytical error.

Attention is also called to the fact that with the protein-poor diet there have been found in the urine subnormal quantities of ammonia, total nitrogen, phosphorus, and organic acids.

¹ Read, B. E., *J. Biol. Chem.*, 1921, xlvii, 281.

² McNeal, M. D., *Am. J. Med. Sc.*, 1922, clxiv, 222.

³ Folin, O., *J. Biol. Chem.*, 1914, xvii, 469.

The daily average is tabulated below :

Patient	Age of Opera- tion	Ht. Cm.	Wt. Kg.	No. of Speci- mens	Ammonia Nitrogen Gm.	Total Nitrogen Gm.	Creatinin Gm.	Creatin Gm.	P ₂ Gm.	Organic Acids (=cc. N/10 NaOH)
*Controls										
L. T.		173	63.0	3	0.3018	10.967	1.7448	0.0	0.7087	159.3
L. C. C.		173	67.5	3	0.1959	14.070	1.9776	0.0	0.6528	292.0
Eunuchs										
L. H. P.	16	176	60.0	19	0.3495	12.865	1.2806	0.0254	0.4378	183.7
T. W. C.	26	163	52.0	10	0.8606	8.622	1.1742	0.0	0.3647	279.6
W. C. H.	18	155	66.5	10	0.6265	9.620	1.4384	0.0	0.4587	110.5
C. Y. S.	18	170	58.0	10	0.5169	9.996	1.1676	0.0	0.5130	121.8

*The controls were laboratory technicians living on a protein-poor diet.

MINNESOTA BRANCH

University of Minnesota, April 1, 1925.

196 (2719)

**Spontaneous nephritis and compensatory renal hypertrophy in
albino rats on diet deficient in vitamin A.**

By C. M. JACKSON.

*[From the Department of Anatomy, University of Minnesota,
Minneapolis, Minn.]*

An epidemic of nephritis occurred in our colony of albino rats (*Mus norvegicus albinus*), which during the past year had been fed a modification of the McCollum-Greenman cereal mixture, as follows: oatmeal, 1500 grams; cornmeal, 1500 grams; whole wheat flour, 1500 grams; flaxseed oil meal, 500 grams; bone meal, 75 grams; sodium chloride, 25 grams; dried milk powder, 250 grams. In addition, raw liver was fed weekly, and fresh cabbage twice a week. City water was supplied *ad lib*.

For a while, the rats seemed to thrive fairly well on this diet, although growth was somewhat retarded and reproduction subnormal. The presence of nephritis was discovered last December, during the course of some experiments on compensatory renal hypertrophy. The nephritis usually appeared chronic in form, though in some cases acute and fatal. Apparently all the rats in the colony were affected. The proportion of milk powder was doubled, fresh milk was added to the mixture, and cabbage fed thrice a week, but there was no improvement.

Sixty-five of the rats above 112 grams in body weight were killed, and the weights of the kidneys were compared with Donaldson's Wistar norm for corresponding body length. In 16 cases, the kidneys were above normal (maximum +63 per cent), and in 49 cases below normal (minimum -35 per cent). In spite of this variation in total kidney weight, the two kidneys of the same rat differed but little in weight. The left kidney weight was slightly less in 43 of the 65 cases, however, the general average being 4.6 per cent below the right (total range -18 to +13 per cent).

The kidneys frequently appeared light brown in color, occasionally slightly mottled. The surface sometimes appeared smooth, but often (especially in advanced cases) irregular, with numerous small granules and depressions. The capsule was readily separable. On gross section, the fresh surface usually appeared pale and translucent, often showing yellowish radial striae in the cortex.

In histological preparations, a peculiar interstitial nephritis was found, apparently beginning near the cortico-medullary junction, and extending variably into the cortex, often along the intralobular vessels. Local infiltrations of nononuclear cells were found in the earlier stages, with variable fibrosis and progressive degeneration of the renal tubules later. The renal (Malpighian) corpuscles appeared relatively resistant. Some of the collecting tubules were apparently obstructed, resulting in characteristic, enormous dilations, up to 1 mm. in diameter, filled with secretion, cellular detritus or pus, and lined by cuboidal or flattened epithelium. In extreme cases, the entire uriniferous tubule, excepting the glomerular region, may be distended.

Ophüls and McCoy¹ found a similar spontaneous nephritis with numerous cysts in 2 per cent of captured wild rats. They also produced a similar nephritis in white rats by subcutaneous injection of uranium nitrate, the controls remaining free from lesions. They considered this cystic renal condition characteristic of the rat, but various investigators have observed somewhat similar cystic dilations in the spontaneous nephritis of the rabbit and guinea pig.

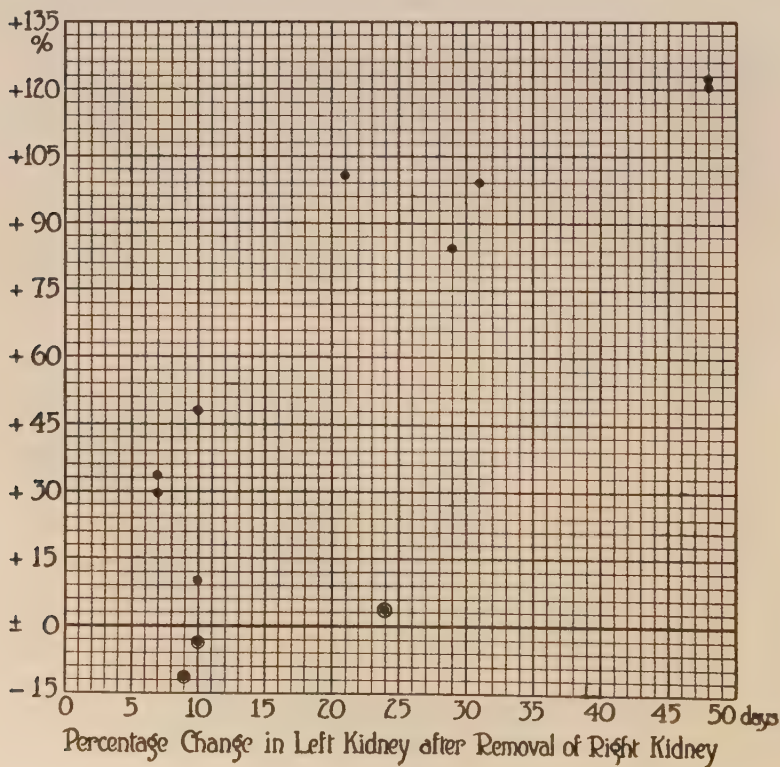
While the immediate cause of the nephritis in the present series was probably an infection, it is possible that a lowered tissue resistance, due to deficient diet, may have been a factor. The diet used appears somewhat deficient in animal protein, and especially in vitamin A. The latter deficiency was evidenced by the frequent appearance of xerophthalmia (conjunctival hemorrhage) in the affected rats. Since vitamin A deficiency is said to favor infections also of the respiratory and alimentary tracts, it may likewise lessen the resistance of the kidney. This might explain the appearance of nephritis in a rat colony in which it had not been found in previous years. It appeared also within two months in a dozen healthy young rats obtained from the

¹ Ophüls, W., and McCoy, G. W., *J. Med. Res.*, 1912, xxvi, 249.

Wistar Institute and put on the same diet, though placed in new cages and kept isolated from the old colony.

The heart and lungs usually appeared normal in the affected rats, the abdominal viscera in general atrophic and devoid of fat. The thorax seemed narrow transversely, with a marked ventral protuberance in the sternal region. Otherwise the skeleton showed no marked gross deformity. The incisor teeth, however, were abnormal, chalky white in color, usually enlarged and irregular, sometimes broken. The upper incisors were often greatly elongated and recurved, failing to articulate with the lower teeth.

In 12 male nephritic rats 8 to 9 months old, weighing 205 to 240 grams, the right kidney was removed (by lumbar incision, under ether anesthesia) and weighed. The same diet was continued, and the wounds healed promptly. In three cases, the rats died in 9 to 24 days, and the remaining left kidney (see en-



circled dots in the accompanying chart) had apparently changed but little in weight. The other 9 rats were killed after various intervals. With one exception, the remaining kidneys showed a progressive increase in weight, varying from about 30 per cent in 7 days to 120 per cent in 48 days. The body weight simultaneously decreased 10 to 38 per cent. Histological study indicated that the increase in renal weight after unilateral nephrectomy was due chiefly to increased dilation of the tubules, but partly to glandular hypertrophy. No change in weight or structure of the kidneys was apparent in 6 control (nephritic) rats kept for corresponding periods after the same operative procedure, but without nephrectomy.

197 (2720)

The relation of learning and retention to the extent of cerebral lesions in the rat.

By K. S. LASHLEY.

[*From the Department of Psychology of the University of Minnesota, Minneapolis, Minn.*]

In a preliminary study of the mass factor in neural function, lesions of various sizes were produced in the visual areas¹ of the cerebral cortex of fourteen rats. The animals were then trained in a habit of brightness discrimination, and the amount of practice required for learning, as indicated by the total number of errors made before the habit was perfected, was correlated with the locus and extent of the lesions. The destructions involved from 13 to 40 per cent of the entire cerebral cortex. The operated animals required an average of 148.4 trials for learning, with an average of 45.6 errors. The average for 60 normal animals in the same problem is 139.7 trials with 41.5 errors. The difference is not significant. All parts of the occipital third of the cerebral cortex were explored in this series, and the rate of learning is apparently unaffected by the locus of the injury. The rank-order correlation between the amount of cortex destroyed and the number of errors made in learning is 0.084. The rate for

¹ Lashley, K. S., *Am. J. Physiol.*, 1922, lix, 44.

initial learning of brightness discrimination, after lesions to the visual area, is independent of the extent of the lesion.

In a second series, twenty-three normal animals were trained in the same habit. When learning was completed, lesions of various loci and magnitude were produced in the occipital cortex. Seven days after operation the degree of retention of the habit was measured in terms of the numbers of errors made before accurate discrimination was re-established. This amount of practice required for relearning was correlated with the locus and extent of the lesions. The injuries involved from 1.5 to 31.6 per cent of the total cortex; the errors made in relearning ranged from 0 to 83. The rank-order correlation between the extent of the lesion and number of errors in relearning is 0.864 ± 0.037 . In the series of animals every possible part of the occipital third of the cortex was destroyed by the smaller lesions with little disturbance to the habit; whereas in the cases with extensive lesions and serious habit disturbance, every part of the occipital cortex escaped injury in one animal or another. The degree of retention is therefore independent of the locus of the lesion, but directly proportional to its extent.

The possibility that the results are due to the general shock of operation is ruled out by the fact that equally extensive injuries in the frontal, orbital, and parietal areas and basal ganglia produce no disturbance of visual discrimination. The high correlation in retention tests, and the lack of any significant correlation in initial learning, indicate that the results are due not to a total blinding of part of the visual field (as in hemianopsia) but rather to some factor of mass action in the central associative mechanism.

Clinical studies of man have indicated that small lesions in the cerebrum may produce no symptoms, and that, especially in the association areas, severe and long continued disturbances of behavior result only from extensive injuries. The present study points to a much greater precision in this quantitative relationship than has been recognized in the clinical literature.

198 (2721)

The accumulation of chlorides in the leaf tissue fluids of Egyptian cotton with the march of the season.

By J. ARTHUR HARRIS.

[*From the Department of Botany, University of Minnesota, Minneapolis, Minn., and the U. S. Department of Agriculture, Washington, D. C.*]

Among the physiological problems presented by the phenomenon of the differential absorption and tolerance in solution of chlorides and sulphates by the Egyptian and Upland types of cotton¹ is that of the change in the concentration of these anions with the march of the season. This problem is in reality two-fold: (*a*) change in the concentration in one and the same organ with its development and senescence, and (*b*) the possible change in the concentrations found in the (leaf) tissues at as nearly as possible comparable stages of maturity at earlier and later periods in the development of the organism as a whole. The present discussion is limited to the latter phase.

The more mature cotton plant does not lend itself readily to controlled laboratory experimentation. For this and other reasons a statistical method of attack seems desirable. The problem presents some difficulties, since concentrations based on unit volumes of tissue fluid may obviously be influenced by (*a*) errors of judgment or the errors of random sampling in the collection of leaves at earlier and later periods,² and by (*b*) the turgidity of the tissues at the time of collection of samples grown in field cultures.

Table I shows the mean concentration of chlorides in terms of grams per liter of leaf tissue fluids from samples from the same plants in a first and second series of analyses³ of the number (*N*)

¹ Harris, J. Arthur, Hoffman, W. F., and Lawrence, J. V., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, xxii, 350-352.

² Both sets of determinations were based on random samples of mature leaves. Slight differences in the maturity of the collections of leaves might influence the averages.

³ In the experiment of 1922 the first series of samples was taken from July 25 to Aug. 9, whereas the second series was taken Aug. 28 to Sept. 4. In the experiment of 1923 the first series was taken July 29 to Aug. 14, whereas the second series was taken Aug. 18 to Aug. 31.

indicated based on cottons grown in the Gila River Valley in southern Arizona.⁴

Since the determinations of the first and second series may be expected to be correlated, because of the influence of the individuality of the plants and of variation in the environmental conditions to which they are exposed, it is necessary to consider the correlation between the first and second determinations in calculating the probable errors of the difference between them. The formula is

$$E_{(2-1)} = .6745 \sigma_{(2-1)} / \sqrt{N}$$

when N is the number of pairs of determinations and

$$\sigma_{(2-1)}^2 = \sigma_1^2 + \sigma_2^2 - 2r_{12} \sigma_1 \sigma_2$$

σ being the standard deviation of the chloride content of the first or second series as denoted by the subscript, and r_{12} the corre-

TABLE 1. Comparison of chloride content of first and second series of determinations on Egyptian and Upland cotton.

	N	Mean chloride content and probable error	Correlation between first and second series	Absolute difference between first and second series		Percentage difference
				Difference and probable error	Diff.	
					E. Diff.	
Experiment of 1922						
Pima Egyptian						
First series	67	4.6716±.0613				
Second series	67	5.2164±.0636	.702	+.5448±.0482	11.29	11.66
Meade Upland						
First series	66	3.1553±.0597				
Second series	66	2.8106±.0515	.801	-.3447±.0359	9.60	10.92
Acala Upland						
First series	69	3.6920±.0455				
Second series	69	3.4638±.0489	.665	-.2283±.0338	5.89	6.18
Experiment of 1923						
Pima Egyptian						
First series	69	2.4239±.0405				
Second series	69	3.2899±.0508	.684	+.8659±.0375	23.11	25.73
Lone Star Upland						
First series	66	1.0341±.0213				
Second series	66	1.1628±.0308	.628	+.1288±.0240	5.36	12.45
Fi Hybrid						
First series	90	0.8333±.0212				
Second series	90	1.1083±.0273	.795	+.2750±.0166	16.61	33.00

⁴ The materials are the same as those considered in another connection by Harris, J. Arthur, Lawrence, J. V., and Lawrence, Z. W., *J. Agr. Res.*, 1924, xxviii, 695.

lation between them. The coefficients in the second column of constants show that there is a rather high correlation between the first and the second determination on the same group of plants. These do not require discussion further than to note that they have been used in determining the probable errors of the differences by the formula indicated above.

The absolute differences show that in the experiments of both years, Pima Egyptian cotton has a materially higher chloride content in the second series than in the first series of determinations. The differences are clearly significant in comparison with their probable errors. They amount to about 12 per cent of the first average in the experiment of 1922, and to about 36 per cent of the average of the first series of determinations in the experiment of 1923.

In the case of the Upland varieties there seems to be a slight but significant decrease in the chloride content (amounting to about 6 and 11 per cent in the two varieties, Acala and Pima) in the experiment of 1922, but a slight increase in chloride content in the Lone Star Upland variety grown in 1923.

The results seem to indicate an increase in the magnitude of the chloride concentration of the plant as a whole with the advance of the season in the case of the Egyptian plants, but to leave such a change open to question in the case of the associated Upland plants.

It is interesting to note that the type (Egyptian) which has been shown⁵ to be characterized by the highest chloride content also shows most clearly an increase in the concentration of this anion with the march of the season.

The single series of determinations made in 1923 on the F₁ hybrid between Pima Egyptian and Lone Star Upland cotton indicates an increase in the chloride content of the hybrid very similar to that in the Upland parent.

These investigations are being continued.

⁵ Harris, Lawrence and Lawrence, *Loc. cit.*

ERRATUM: In Dr. A. A. Horvath's paper [97(2620) December, 1924] on "The Action of Ammonia upon the Lungs," "per cents" should read "per mille".

SCIENTIFIC PROCEEDINGS.

NEW YORK MEETING

Columbia University, New York City, May 20, 1925.

199 (2722)

The functional activity of the breast in relation to mammary carcinoma in mice.

By HALSEY J. BAGG.

[From the Memorial Hospital and Cornell University Medical College, New York City.]

The following experiments were designed to test the possible relation of functional disturbances of the breast to the incidence of mammary carcinoma in mice.

A carefully inbred strain of animals with a well-known low rate of tumor incidence was selected. Mammary tumors in females from this strain that are allowed to breed normally do not appear until the animals are between one and a half to two years of age. The females were large, vigorous, prolific individuals, and suckled their young successfully.

Females from the above strain were divided into two groups, one serving as a control was allowed to breed normally, *i. e.*, the females were remated only after nursing their litters for about six weeks. The test animals from the same family were then bred under the following procedures designed to subject the mammary tissues to abnormal conditions:

1. Females bred when very young (two to three months old), and their litters removed immediately after parturition, or not more than twelve hours later, and the mothers remated at the oestrus that promptly follow parturition.
2. Females bred when six to nine months old, and treated as in the above group.

3. Various combinations of alternating periods of suckling of one litter and non-suckling of the next litter, etc.

4. Ligating mammary ducts to the nipples on one side of the body when the females were about half way through the period of pregnancy, followed by suckling of litter and remating after weaning. The operative trauma was minimized as much as possible. No attempt was made to prevent suckling on the unoperated side of the body.

The results are based on the records of fifteen females with spontaneous tumors, all of which have been found to be mammary carcinoma on histological study.

Animals of Group 1 have bred very rapidly, having had as many as nine to eleven consecutive litters, with an average of only twenty-four days between the dates of parturition. In this group mammary tumors have appeared at an early age, *i. e.*, when the females were from eight to eleven months of age.

In Group 2 the age of the females at the time of tumor incidence is from eleven to twelve months. In this group a relatively low average of only four periods of pregnancy, followed by the prevention of suckling, precedes the onset of the tumors.

Animals of Group 3 show on the whole that, when periods of suckling alternate with periods of non-suckling, the tumors appear after fewer consecutive litters, as compared with the condition in Group 1.

Prevention of proper drainage of the breast has resulted in marked stasis of the mammary ducts. This is associated with the presence in the mammary gland of a considerable amount of decomposing, stagnated and probably chemically altered milk, which it is possible was sufficiently irritating to cause considerable disturbance within the breast.

Contributory evidence was given to this view by the histological study of serial sections of a considerable portion of the breast in mice with very early but definitely established mammary carcinoma. The cyclic relation between ovarian and mammary systems was obviously deranged by the experimental methods employed, and the breasts in rapidly breeding, non-suckling females were not able to properly reach a normal resting condition for any considerable period of time.

In mice breeding rapidly from an early age (Group 1) and not allowed to suckle their young, the breasts were apparently pre-

vented from reaching their normal degree of development; and it took a considerable number of consecutive litters, followed by periods of non-suckling, before the onset of tumor growth. Whereas, in animals older when first bred (Group 2) or in Group 3, where suckling at one period definitely established a large, well-developed breast to start with, it took fewer periods of pregnancy followed by non-suckling before the tumors became evident.

The tumors have appeared coincidentally with, or a day or two after, parturition. The animals have grown tumors and embryos at the same time.

Mammary ducts have been ligated with the consideration in mind of the possible significance of mammary duct stasis in relation to tumor growth. This group of animals contains only a few individuals, but nevertheless it is interesting to note that in the first animals tested within the last few weeks, two of the first females operated upon developed mammary tumors on the side of the body on which the ducts had been ligated.

Histological examination showed the presence of solid mammary carcinoma, associated with marked stasis of the duct system on the ligated side of the body.

The tumors that appeared in the four groups of experimental animals were definitely malignant in nature, with a rapid rate of growth and a marked tendency to local recurrence after operative removal. The breasts have usually shown evidence of multiple foci of tumor growth.

In the control group of animals containing females breeding regularly and suckling their young, the incidence of tumor growth is less than 5 per cent, and the tumors make their appearance only in animals between about eighteen to twenty-four months of age. Although this report is designed mainly as a preliminary report, and further data will be added in the near future, it has been thought sufficiently of interest to state now, that in the first group of fifteen animals, in which a dysfunction of the breast was brought about by non-traumatic means, thirteen females or eighty-seven per cent developed mammary carcinoma at comparatively early ages as described above.

The presence of the two females that developed mammary carcinoma when only six months of age following ligation of mammary ducts, means nothing when viewed alone, but is of interest in connection with the above mentioned groups.

200 (2723)

The reactivation of inactivated insulin *in vitro* and *in vivo*.

By ALBERT A. EPSTEIN.

[From the Laboratory of Physiological Chemistry, Pathological Department, Mt. Sinai Hospital, New York City.]

Studies previously reported^{1, 2} have established the fact that trypsin can inactivate insulin both *in vitro* and *in vivo*. It has also been ascertained that dissociation of insulin³ from its inactive combination with trypsin is possible *in vitro* by proper adjustment of the hydrogen ion concentration of the substance in solution, *i. e.*, by shifting the pH to the acid side of 4.6. It became desirable to ascertain whether or not dissociation of insulin from trypsin could be effected *in vivo*. It is obvious that the hydrogen ion concentration of the body fluids could not be altered to the point necessary for the dissociation of insulin. Other means were therefore tried, and the following results obtained:

1. Upon the addition, *in vitro*, of such substances as pepsin, safranin and cryogenin (M-Benzaminosemicarbazide) to the solutions of inactivated insulin (trypsin), liberation of the insulin takes place. This is evidenced by the result of injection of these mixtures into suitable test animals.

2. These agents (pepsin, safranin and cryogenin) can cause dissociation of insulin from trypsin directly *in vivo*. Injection of adequate amounts of these substances into animals (subcutaneously or intravenously) made just prior to the parenteral administration of inactivated insulin, cause liberation of the insulin and the production of its physiological effects.

The accompanying tables illustrate the effect on the blood sugar of one of the reactivating substances used:

¹ Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1924, lxx, 225.

² Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1925, lxxi, 316.

³ Epstein, Albert A., Rosenthal, Nathan, and others, *Am. J. Physiol.*, 1924, lxx, 225.

TABLE I.

Showing the effect on the blood sugar of inactive insulin and of reactivated Insulin.

Animal No. 67. Rabbit, weight 1.5 kgm.

Blood sugar examination	Inactivated Insulin* Insulin + Trypsin	Reactivated Insulin Insulin + Trypsin preceded by Cryogenin
	Per cent	Per cent
Before injection	0.125	0.129
15 min. later	0.131	0.125
30 min. later	0.120	0.070
45 min. later	0.130	0.080
60 min. later	0.134	0.063
75 min. later	0.135	0.070 (Convulsions)

Animal No. 68. Rabbit, weight 1.4 kgm.

Before injection	0.126	0.130
15 min. later	0.115	0.120
30 min. later	0.135	0.105
45 min. later	0.140	0.090
60 min. later	-----	0.078
75 min. later	0.143	0.095

TABLE II.

Showing the effect on the blood sugar of insulin, insulin and the reactivating substance, and of the reactivating substance alone.

Animal No. 65. Rabbit, weight 1.5 kgm.

Blood sugar examination	Insulin†	Insulin and the Reactivating Subst. (Cryogenin)	Reactivating Subst. (Cryogenin)
	Per cent	Per cent	Per cent
Before injection	0.135	0.122	0.140
15 min. later	0.133	0.140	0.155
30 min. later	0.115	0.090	0.145
45 min. later	0.107	0.110	0.145
60 min. later	0.106	0.100	-----
75 min. later	0.112	0.120	-----
90 min. later	0.080	-----	-----
105 min. later	-----	-----	-----
120 min. later‡	-----	0.125	-----

*All the injections were made intravenously.

†One unit of insulin per kgm. body weight was used.

‡Animals were observed for 4 hours more, and when no convulsions developed they were fed.

201 (2724)

Effect of insulin on the metabolism of dogs under amytal anesthesia.

By HARRY J. DEUEL, JR. and WILLIAM H. CHAMBERS with the technical assistance of JAMES EVENGEN.

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

The effect of insulin on the metabolism of normal fasting dogs has been studied in the respiration calorimeter for four hour periods after its injection. Control experiments were carried out to determine the effect of amytal anesthesia on the heat production of the dog. This anesthetic has no effect on blood sugar¹ and we have found that it has no effect on the CO₂ combining power of the blood.

When sufficient amytal was injected to produce a complete anesthesia (doses from 55 to 65 mg. per kilo, depending on the individual animal), the metabolism remained constant over a six hour period at a level of less than 5 per cent below the basal. On the production of deeper anesthesia by larger doses of this hypnotic, a larger decrease in metabolism occurred (10-25 per cent), but this usually remained constant over a six hour period provided no shivering resulted.

A marked fall in body temperature occurs following the amytal anesthesia which is very rapid at first, and the body temperature usually reaches the minimum value in the second or third hour. With light anesthesia shivering usually resulted when the minimum temperature was reached, by means of which the metabolism was markedly accelerated and the body temperature brought back to normal. With larger doses of amytal, chemical regulation was entirely prevented, although a slow return to normal temperature resulted.

When insulin was injected into a fasting dog under the influence of amytal anesthesia, an increased R. Q. was sometimes obtained during the second hour, while during the third and fourth hours the R. Q. returned to the preliminary level. In other experiments on the same animals no increase in quotient

¹ Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 194.

followed the administration of insulin, although the blood sugar fell decidedly below the convulsive level.

A progressive increase in heat production usually occurred after the administration of insulin irrespective of the R. Q. obtained. This may indicate that insulin directly increases fat metabolism.

202 (2725)

Disappearance of ketone bodies in presence of unoxdized sugar in completely phlorizinized dogs.

By M. WIERZUCHOWSKI. (Introduced by Graham Luske).

[*From the Physiological Laboratory, Cornell University Medical College, New York City.*]

Sugar (glucose or levulose), given in proper amounts to starving, completely phlorhizinized dogs which have ketosis and are in coma, causes the disappearance of ketone bodies from the urine and blood for a period of 9 to 24 hours with corresponding clinical improvement of the animal, in spite of the fact that the sugar given may not be oxidized as has been shown by calorimeter experiments,¹ and may be quantitatively recovered from the urine. The degree of aketosis (the state when ketosis is absent when it could be expected) is favorably influenced by the amount of sugar administered, and therefore by the curve of extra sugar excretion. The excretion of ketone bodies after administration of sugar goes hand in hand with the fall in the nitrogen elimination noted by Deuel and Chambers.² The ketone bodies do not stagnate in the body because they disappear from the blood. There is no increase of the ketosis symptoms, but in some cases an amelioration of these symptoms; and there is no compensatory after excretion of the ketone bodies. There is also no renal impairment in Allen's sense³ which could cause aketosis. There

¹ Ringer, Michael, *J. Biol. Chem.*, 1923, lviii, 483.

² Deuel, H. J., Jr., and Chambers, Wm. H., *J. Biol. Chem.*, 1925, lxiii, 22.

³ Allen, F. M., *J. Metabol. Res.*, 1923, iv, 579.

is no difference between glucose and levulose in producing aketosis.

Thus the "fire of carbohydrates" is not always necessary to oxidize completely the end products of fat metabolism in the phlorhizinized dog, but a mere increase in the quantity of glucose is sufficient to do so, possibly, by contact action between it and the ketone bodies. From a general standpoint it is interesting to note that a substance, such as sugar, which is not retained nor oxidized but is slowly and completely eliminated from the body, nevertheless, by its mere presence in even small amounts (as little as 20 grams) is able to produce a complete oxidation of fat to its normal end products, associated with a sparing action upon protein, and at the same time to improve, sometimes to a high degree, the clinical state of the dogs in diabetic coma.

203 (2726)

Ramon flocculation test for determining potency of antiscarlatinal serum.

By OLGA R. POVITZKY. (Introduced by Anna Williams).

[*From the Bureau of Laboratories, Health Department,
New York City.*]

The Ramon flocculation test has proven quite successful for determining the potency of antitoxic diphtheria serums. It is used now at the Research Laboratory of the City Health Department for the routine study of the progress of diphtheria horses; these tests need to be verified only here and there by guinea pig inoculations.

The above results with anti-diphtheritic serums led to attempts to apply the flocculation test, if possible, for determining the potency of antiscarlatinal serums, as compared with clinical neutralization tests.

For titration of the anti-scarlatinal serum, or of toxin, we rely on skin reactions. The strength of the toxin is expressed by the number of skin doses per cc. required for a reaction; and the potency of the serum is estimated by the amount of serum required to neutralize the number of skin doses per cc. Thus if

the titrated toxin contains 40,000 skin doses per cc. and flocculation occurs with 2 cc. of the tested serum, we would estimate that the serum contains 20,000 neutralizing skin doses per cc. A table can be then constructed, with a titrated toxin, in the same manner as for the diphtheria Ramon tests, on the basis of units. Thus in the above cited case the serum contains $\frac{1}{2}$ unit per cc.; if one cc. of serum were required for neutralization, the serum would contain 1 unit; if $\frac{1}{2}$ cc. is used, 2 units, etc.

Though the flocculation tests for scarlet fever serum were undertaken many months ago, no visible results were obtained until lately. The explanation is possible, that though we had good toxins, we did not have strong enough serums to make the test workable in the test tube. At present our own horses as well as the horses from different laboratories have furnished us with stronger serums, and the potency of some of them can be demonstrated by flocculation test, and compared with the skin neutralization tests in humans.

The favorable results thus far obtained are: Our own scarlatinal serum horse No. 50, whose serum continued negative for a long time by the flocculation test, shows in the last successive bleedings, flocculation with amounts from 4 cc. to 2 cc. with one cc. of toxin containing 40,000 skin doses per cc. This closely corresponds to the skin reactions. Corresponding results were obtained with two other weaker toxins. Four sera from outside laboratories which were labelled "15,000 neutralizing doses per cc." gave flocculations with the above 1 cc. of toxin in amounts from 3 cc. to 2 cc. of serum. One serum also from an outside laboratory gave an instant flocculation with anti-scarlatinal, with diphtheria toxin, as well as with plain broth, though the control of the serum itself remained clear. It is possible the preservative used in the last serum gave the non-specific reactions. Controls were also made with anti-streptococcus serum and a few normal serums, with negative results. On the other hand almost every anti-scarlatinal horse serum showed flocculation with diphtheria toxin, when the serums used were in large amounts (from 5 to 3 cc.) These amounts of serum would detect from $\frac{1}{4}$ to $\frac{1}{3}$ unit of diphtheria antitoxin per cc. Such small amounts of diphtheria antitoxin is normal for horses, especially as some might have been old diphtheria horses, and the specificity for scarlet fever may not necessarily be questioned.

The above cited tests though encouraging are by no means conclusive or definite as yet. Much more work must be done to be sure of the specificity of the flocculation test for scarlet fever serums and toxins, as in the case of diphtheria.

204 (2727)

Note on the relationship between insulin and trypsin.

By ERWIN BRAND and MARTA SANDBERG. (Introduced by David Marine).

[From the Division of Laboratories, Montefiore Hospital,
New York City, N. Y.]

Epstein¹ and his co-workers have recently claimed that insulin is not digested by trypsin and pepsin. It is supposed to form an inactive addition product with trypsin, which is stable at an alkaline pH, and from which the insulin can be reactivated by acidifying. A similar reaction occurs with pepsin, but under opposite conditions as far as the pH is concerned. The physiological and clinical aspects of these *in vitro* experiments were followed up by Epstein² and his co-workers by *in vivo* experiments and perfusion studies of the pancreas.

Epstein's results, however, as far as the perfusion experiments go, were contradicted by W. S. Collens,³ and his *in vivo* experiments could not be supported by D. A. Scott.⁴ As to Epstein's *in vitro* experiments, Scott was able to confirm that insulin forms an inactive addition compound with trypsin, from which it can be reactivated. But he found, in accord with previous investigators, that trypsin digests insulin. The fact that acid reagents reactivate insulin from its inactive addition compound with trypsin, led Scott to point out other results which indicate that insulin may exist in the pancreas and in the body in an inactive form.

¹ Epstein, A. A., and Rosenthal, N., *J. Am. Med. Assn.*, 1924, lxxxii, 1990; Epstein, A. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, xxii, 9; Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1924, lxx, 225.

² Epstein, A. A., and Rosenthal, N., *Am. J. Physiol.*, 1925, lxxi, 316.

³ Collens, W. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, xxii, 367.

⁴ Scott, D. A., *J. Biol. Chem.*, 1925, lxiii, 641.

The finding that more insulin is extracted from tissues by acid reagents than by water cannot be taken as evidence that insulin exists in the tissues in an inactive form. This phenomenon is a consequence of the chemical properties of amphoteric colloids.

Scott suggests that the primary action between trypsin and insulin is a mutual adsorption phenomenon, which is followed by complete proteoclastic destruction. He states that this is in accord with the theory of enzyme action, referring to the textbook of Bayliss.⁵ Bayliss, from a colloidchemical point of view, supports the idea of enzyme and substrate forming an adsorption compound, and bases their relation on the so-called adsorption-isotherm. Diametrically opposed to this view are the more recent views of the majority of investigators who consider the reaction between enzyme and substrate as of a purely chemical nature following the mass law. For those who do not accept the view of Bayliss, the theory of Scott is also not acceptable.

We were interested in the problem of the insulin-trypsin reaction from quite a different point of view from that of Epstein and Scott. In this connection we should like to mention a few experiments which were carried out about a year ago. Recently, Willstätter and Waldschmidt-Leitz were able to decide between the different theories on the action of enterokinase on trypsin, in favor of the so-called complement theory. Waldschmidt-Leitz showed definitely, confirming previous investigators, that the reaction between kinase and trypsin involves the formation of a loose addition compound, and that this combination follows stoichiometrical laws. These interesting results and the inexplicable phenomenon of certain inhibitors of the tryptic system which are present or formed in the pancreas, induced us to study the influence of insulin on tryptic digestion. We used in our experiments a trypsin preparation prepared from dried pancreas according to Willstätter,⁶ which already showed the so-called spontaneous activation. We found no change in the tryptic activity⁷ either in the presence of insulin or after previous treat-

⁵ Bayliss, W. M., *The nature of enzyme action*, New York, 3rd edition, 1914.

⁶ Willstätter, R., and Waldschmidt-Leitz, E., *Ztschr. f. physiol. Chem.*, 1923, cxxv, 132.

⁷ In our experiments we used the method of Willstätter and Waldschmidt-Leitz, *Ztschr. f. physiol. Chem.*, 1924, cxxxii, 181. This method, however, has to be modified according to the latest results of Willstätter, cf. Willstätter, R., and Persiel, H., *Ztschr. f. physiol. Chem.*, 1925, cxxxxii, 245.

ment with insulin at different hydrogen ion concentrations. At that time we saw no necessity for publishing these results.

Epstein's first communications caused us to take this question up again. While the interpretation of our experiments seemed to permit the formation of an addition compound between insulin and trypsin, it did not agree with Epstein's finding that trypsin does not digest insulin. The formation of an insulin-trypsin compound without subsequent digestion would mean that the combination between trypsin and insulin is of a different nature from the combination between trypsin and gelatine, or trypsin and casein. In this case, according to theoretical considerations,⁸ insulin might be expected to act as an inhibitor of tryptic digestion, while, in our experiments, it did not act in this way. In the first place our preliminary experiments showed, in accord with Scott, and contrary to Epstein, that trypsin digests insulin. This result eliminates the necessity of assuming that the trypsin-insulin compound is of a different nature from the trypsin-gelatine compound.

Many of the earlier investigators as well as more recent authors like Michaelis, Euler, Willstätter and Kuhn consider the intermediate compound between enzyme and substrate the necessary base for enzyme action. Northrop,⁹ however, in a series of exact and mathematically analyzed investigations on pepsin and trypsin has found that his results could not be accounted for by the assumption of an intermediate compound between enzyme and substrate.

The insulin-trypsin and insulin-pepsin reactions now seem to furnish the first biological evidence of the existence of the intermediate compound between enzyme and substrate. It is significant that the intermediate compound between enzyme and substrate in the case of trypsin and insulin is apparently formed involving the physiologically active group of insulin.

Summary.

1. Insulin combines with trypsin not as an inhibitor of trypsin.
2. Trypsin combining with insulin inhibits the action of the

⁸ cf. Michaelis, L., and Rona, P., *Biochem. Ztschr.*, 1914, lx, 62.

⁹ Northrop, J. H., *J. Gen. Physiol.*, 1922, iv, 487; Northrop, J. H., *Die Naturwissenschaften*, 1923, xi, 713; Northrop, J. H., *J. Gen. Physiol.*, 1924, vi, 239.

latter at first reversibly and later irreversibly, probably by digestion.

3. It is possible that further study of the nature of the insulin-enzyme reaction will help to decide whether proteolytic enzymes can act without the formation of an intermediate compound between enzyme and substrate.

205 (2728)

Influence of Ultra-violet radiations on basal metabolism in children.

By MARGARET E. FRIES. (Introduced by B. Schick).

[*From the Pediatric Service of Mount Sinai Hospital, New York City.*]

When malnourished children are exposed to ultra-violet radiations, there is often an improvement in their appetite and a gain in weight. The cause for this phenomena has not been satisfactorily explained. Until recently the impression has been that there is a concomitant rise in the basal metabolism. Otto Kestner¹ and his co-workers have demonstrated that there is an immediate rise in the basal metabolism, which, as far as they investigated, remains elevated for only a short time. Therefore the present work was undertaken to ascertain whether this rise in the basal metabolism extended over a long period of time, and whether it bore any relation to the clinical results.

The investigation was carried on from the beginning of December to the end of March, a period when the intensity of the ultra-violet rays from the sun is not sufficiently strong to influence results. Three children on the Pediatric Service of the Mount Sinai Hospital were studied. Two were free from all organic defects, while the third had a simple goitre. To obtain comparative figures these children were observed in the hospital for a control period of one month. They were on a fixed calculated diet, and their basal metabolism was determined three times

¹ Kestner, Peemoller, Plaut, *Klin. Therap. Wehnschr.*, No. 44, Oct., 1923, p. 2018.

each week. The children were then subjected to ultra-violet radiations from a Hanovian Quartz Mercury lamp. The routine and diet were continued as before. Basal metabolism determinations were again made three times a week, each determination following the light treatment by twenty to seventy hours.

Case 1 received twenty treatments within twenty-four days. Case 2, due to the isolation of the ward, at first received treatment at irregular intervals. The first four treatments were given within one month, and the last eight within nineteen days. Case 3 received seven treatments within sixteen days. The duration of each exposure and the distance of the lamp varied with the individual's reaction. At first the distance of the lamp was twenty-four inches, and gradually decreased to sixteen, while the time of exposure was increased from three to fifteen minutes (anterior and posterior). All children became pigmented.

The Krogh basal metabolism apparatus was used, and the calculations were made from tracings. Three determinations were made each time and the final calculation was based on the lowest reading.

The results showed that there was no appreciable difference in the basal metabolism rate during the months of treatment and non-treatment. The variations of each basal metabolism determination were negligible. Comparing the average basal metabolism before and after treatment, there was no greater variation than two calories per kilo per twenty-four hours.

During the same period, two healthy ambulatory children from the out-patient clinic were observed and similarly treated. They returned three times a week for ultra-violet radiations, and once a week for basal metabolism determination. (Benedict apparatus was used.) The basal metabolism of one case remained stationary, while that of the other child fell twenty-four per cent after two series of treatments within three months. As their diet could not be controlled no definite conclusions could be drawn from these two cases.

The effect of ultra-violet radiations upon basal metabolism is probably dependent upon the individual's reaction to the rays. No increase was noted in the basal metabolism of these five children, nor was there any increase observed in that of two other children studied and treated at the New York Infirmity for Women and Children last April and May. In spite of the inten-

sity of the ultra-violet rays of the sun at that time of the year, which might have influenced the results, the basal metabolism of both children did not rise permanently. Although it rose after the fifth and third treatment, respectively, it then fell, the one case 11 per cent below the original level, and in the other thirteen per cent.

Conclusions: It is important to bear in mind the reactions of the individual, when considering the effect of ultra-violet rays on the basal metabolism. A prolonged rise in basal metabolism following ultra-violet irradiation, is apparently not the usual reaction, but may possibly be found in certain children. The explanation must be sought elsewhere, to account for the clinical improvement noted in malnourished children following irradiation.

206 (2729)

Observations on lytic principle of weak potency.

By GREGORY SHWARTZMAN. (Introduced by Israel S. Kleiner).

[*From the Laboratory of Bacteriology, N. Y. H. Medical College and Flower Hospital, New York City.*]

Bordet and Ciuca¹ have demonstrated that an anticolon lytic principle of weak potency can be obtained from a strong one by addition of a very small amount of strong principle to *B. coli*. The quantitatively reduced lytic principle produces only slight lysis, and according to these authors induces generation of a *new* principle of weak potency. This transformation of a strong principle into a weak one is considered of a qualitative nature, since no matter what amount of this weak principle is added to *B. coli* only weak lysis occurs and a weak principle is generated. To explain these observations Bordet and Ciuca assume that the introduction of a quantitatively reduced lytic principle leads to its considerable dispersion among the bacterial cells. Therefore, each bacterial cell is only weakly impressed by this dispersed

¹ Bordet and Ciuca, *Compt. Rend. Soc. de Biol.*, 1922, lxxxvi, 295, and 1922, lxxxvii, 366 and 987.

principle, and thus reacts with generation of a new qualitatively reduced lytic principle. The technique for producing this variation in the quality of the principle, as given by Bordet and Ciuca, is as follows: Highly diluted lytic principle is added to broth, and inoculated with *B. coli*. In 24 hours only weak lysis occurs. This culture is incubated for about one week, and then sterilized by heating at 58° for one half hour. This fluid when added to *B. coli* produces slight lysis. If the second culture is then left in the incubator for one week and subsequently sterilized by heating, it shows again the presence of a weak lytic principle.

It is apparent from the description of the method employed that incubation for about one week, of cultures showing slight lysis is a necessary step for obtaining a weak principle. However, if the explanation of the mechanism of formation of weak lytic principle given by Bordet and Ciuca is correct, the prolonged incubation of the cultures mentioned above should not be necessary. It is evident that, according to the conception of these authors, weakly impressed bacterial cells should give rise to *only* a weak principle, and that at no time should these cultures show the presence of a principle of strong potency.

The object of this work was to investigate the relation of prolonged incubation of a quantitatively reduced lytic principle to the formation of a principle of weak potency. A series of experiments led to the following results:

I. When the lytic principle is highly diluted (10^{-8}) and inoculated with *B. coli*, only weak lysis occurs in the culture.

II. If this culture is sterilized by heating to 58° for one half hour 24 hours after inoculation with *B. coli* and tested immediately for its lytic potency, it shows the presence of a *qualitatively unchanged* but *quantitatively weaker* lytic principle than the original one. In other words, if a series of dilutions of this principle is made, it is able to produce strong lysis in a dilution not higher than 10^{-6} and slight lysis in dilution 10^{-6} , while the original principle produces strong lysis, does so in dilutions up to 10^{-7} .

III. If the same culture showing weak lysis is left in the incubator at 37° for one week, and then, after sterilization by heating to 58° , is diluted in tubes of broth inoculated with *B. coli*, it shows the presence of a weak lytic principle. That is

to say, it produces slight lysis in dilutions up to 10^{-6} , and has no effect whatsoever in higher dilutions.

IV. If the same culture to which reference is made in paragraphs "I", "II", and "III" is heated to 58° 24 hours after inoculation with *B. coli*, incubated for one week and then tested for its lytic potency, it shows the presence of a weak lytic principle producing slight lysis in dilutions from 10^{-1} up to 10^{-6} , and no lysis in higher dilutions.

V. However, if such a weak lytic principle as is mentioned in paragraphs "III" and "IV" is brought in contact with *B. coli*, it induces generation of a principle of strong potency again.

It is evident from the results obtained that a highly diluted lytic principle induces the generation of a qualitatively unchanged but quantitatively reduced lytic principle. Such a principle² undergoes partial inactivation on standing in the incubator, but no qualitative variation occurs in it since it can be regenerated to full strength.

CONCLUSIONS.

1. The formation of a weak principle is brought about by the partial inactivation of a quantitatively reduced lytic principle, which partial inactivation is produced by prolonged standing in the incubator, and is not, as held by Bordet and Ciuca, the result of a quantitative reduction by itself.

2. No qualitative transformation of lytic principle occurs if the method indicated by these authors is employed.

² No noticeable inactivation of original undiluted lytic principle on prolonged standing in the incubator was ever observed.

207 (2730)

The diagnosis of early human pregnancy by the vaginal smear method.

By GEORGE N. PAPANICOLAOU.*

[*From the Department of Anatomy, Cornell University Medical College and the Woman's Hospital, New York City.*]

The study of a large number of vaginal smears from different mammals, especially from guinea pigs, during the last several years has led me to realize that pregnancy as well as different pathological conditions of the ovaries and the genital tract might be diagnosed more or less accurately by such smears. The entire composition of the vaginal smear changes rather typically under different conditions. Pregnancy, cystic or other degenerative changes of the ovaries, inflammatory processes, growths, etc., affect the entire genital tract, including the vagina, in a way which produces definite and typical changes in the consistency and make-up of the vaginal smear. The presence or absence of different types of desquamated cells, as well as the varying form and number of leucocytes, lymphocytes and erythrocytes and bacteria, offer a variety of criteria upon which a diagnosis of certain conditions may be based.

Since diagnostic reactions are of more interest and importance in man than in other mammals, I have tried for some time to secure proper human material on which to further these studies. It had been difficult, however, to obtain good reliable human vaginal smears until recently. This has now been rendered possible by a coöperative arrangement between the Department of Anatomy of Cornell Medical College and the Woman's Hospital of New York aided by the Maternal Health Committee.

This arrangement has afforded the opportunity to study a large number of human vaginal smears from normal cases as well as from cases of pregnancy, and from several pathological conditions. The normal human smears differ considerably in cellular composition from those of lower mammals, yet they nevertheless show typical and characteristic changes which may serve to indi-

* Aided by a grant from the Committee for Research on Sex Problems of the National Research Council. Grant directed by Dr. C. R. Stockard.

cate a number of distinct stages. The ovulation moment itself is revealed by certain modifications in the smear which will be fully described in another paper. A subnormal condition of the ovary may be revealed or indicated by the absence of those reactions which characterize a normally functioning and ovulating ovary.

The occurrence of pregnancy may be definitely recognized by certain typical changes in the structure of the smear. The prevailing types of desquamated cells show during pregnancy a deviation from the usual types. Soon after the beginning of pregnancy there is a distinct tendency for the vaginal cells to assume certain characteristic forms. Many of them are elongated and concave. The cytoplasm is often partly or totally plasmolyzed or vacuolized. Some are more or less collapsed and appear boat like in shape. The nucleus is often also elongated and collapsed. Such cells may appear occasionally under normal or pathological conditions, but not so typically and steadily characteristic as in the pregnancy smear. The diagnosis of pregnancy, however, need not be based solely upon the presence or absence of such cells. Other characteristics, such as the absence of normal periodicity, the conditions of leucocytes, lymphocytes, erythrocytes and of the other types of cells, may be used in correlation with the above described cellular changes for the complete picture in a definite diagnosis. A further common characteristic of the pregnancy smear is the frequent presence of free nuclei and traces of broken cells.

An important fact is that typical reactions and changes characterize the vaginal smear during both uterine and ectopic pregnancies. This has been definitely established by several ectopic cases diagnosed as pregnant and later operated upon at the Woman's Hospital. The approach or occurrence of parturition, abortion or resorption may possibly also be revealed by certain smear changes. As soon as delivery, abortion or resorption takes place, and the *corpus luteum* begins to regress, the smear presents an entirely different appearance, indicating the onset of active destructive processes.

As a whole, the study of the vaginal smear seems to be of important diagnostic value. Its application may be very helpful in gynecology for the diagnosis of certain doubtful cases. The technic does not offer great difficulties and might be simplified so as to be used as a routine method.

208 (2731)

Hydrogen-ion concentration in the gastro-intestinal tract of the albino rat.

By E. M. ABRAHAMSON and E. G. MILLER, Jr.

[*From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons, New York City.*]

In order more accurately to interpret various findings in nutritional studies with albino rats, it was desired to ascertain the normal alimentary conditions of the rat, with special reference to the hydrogen-ion concentration.

Adult male rats were fed various diets, for periods of from one to nine days, except when otherwise noted below. The food was given *ad lib.*, and the rats were killed several hours after the last day's supply of food had been placed in the cage. The contents of the stomach were promptly removed and filtered through glass wool. The small intestines were divided into two portions of equal length, and the contents gently expressed and filtered. (The scant volume of the intestinal contents made it impracticable to make definite comparisons of the various anatomical divisions of the intestines.) The pH of each fluid was then determined colorimetrically, using the Clark and Lubs indicators, and using standard phosphate solutions which had been checked electrometrically. When necessary, a comparator was used in matching colors.

The diets used were: our standard mixed diet (which includes meat, milk, cheese, corn, rye, oats, bread, cabbage); lean beef alone; beef fat alone; boiled potato alone; Pappenheimer and Sherman's rachitic diet "84" (flour 95 per cent, calcium lactate 2.9 per cent, sodium chloride 2 per cent, ferric citrate 0.1 per cent); and the Pappenheimer-Sherman diet plus five per cent cod liver oil.

In the rats on the mixed diet, and on lean beef, fat, and potato diets, we found varying degrees of acidity throughout the tract, as summarized in the tables below.

In the experiments on the Pappenheimer-Sherman rachitic diet, eight rats were used. After seven weeks on this diet, four of the rats were killed and their gastric and intestinal contents

studied. The other four were continued on the same diet for two more weeks, and then fed the same mixture to which 5 per cent cod liver oil had been added. After two to three weeks of this diet they were killed and studied. As will be seen from the table below, the rachitic rats showed a marked increase in the pH of the intestinal contents (rising as high as 7.4); while the curative effect of cod liver oil was associated with a return to the normal acidity. The rise in pH, with the resulting formation of the insoluble calcium phosphate in the intestine, is probably an important factor in the deficiency of calcium absorption and deposition on the "rachitic diet".

No. of rats	Diet	pH gastric contents	pH upper half int.	pH lower half int.
6	Mixed	3.4, 2.7, 3.4 2.6, 3.2, 2.9	6.0, 5.9, 5.2 5.2, 5.3, 5.2	6.0, 5.9, 5.4 5.2, 5.3, 5.3
6	Lean beef	3.7, 3.8, 3.6 3.4, 3.7, 3.5	5.3, 5.7, 6.0 5.9, 6.1, 6.0	5.4, 5.5, 5.8 5.8, 6.1, 6.0
12	Fat (beef)	2.6, 3.2, 3.2 2.8, 3.6, 3.0 3.3, 3.7, 4.0 3.0, 3.2, 3.5	6.0, 5.2, 5.6 5.9, 5.6, 5.7 6.3, 5.9, 6.4 5.8, 6.0, 6.0	6.1, 5.4, 5.9 6.3, 5.9, 5.7 6.3, 6.0, 6.5 5.9, 5.9, 6.0
6	Potato	3.4, 4.1, 3.4 3.3, 3.0, 3.1	6.0, 6.1, 6.0 6.0, 6.0, 6.0	6.2, 6.1, 5.8 6.0, 6.0, 6.0
2	Fast 24 hrs., then cheese <i>ad lib.</i>	3.8, 3.8	5.9, 6.0	6.1, 6.2
2	Fast 24 hrs., then rye and carrots	3.6, 3.7	6.0, 5.9	6.0, 6.0
2	Fast 24 hrs., then rye and cheese	3.8, 3.8	5.9, 6.0	6.0, 6.0
4	"Rachitic" diet, Pappenh.-Sherman	3.8, 3.8, 3.8 4.0	6.4, 7.0, 7.1 7.0	6.7, 7.0, 7.4 7.0
4	"Rachitic" diet plus cod liver oil	3.2, 3.3, 3.0 3.4	5.4, 5.4, 5.6 5.7	5.6, 5.4, 5.8 5.8

The solid nature of the contents of the large intestine did not permit direct application of the above methods; moreover, the reaction of the colon and feces have been studied by other workers. In 13 of our rats on various diets, the contents of this portion of the intestines was pressed against filter paper, and the moist spot so formed was found to be uniformly violet (alkaline) to bromthymol blue.

209 (2732)

The rôle of the accelerator nerves in bulbar anemia.

By HELEN C. COOMBS.

[From the Department of Physiology, New York University and
Bellevue Medical College, New York City.]

In a previous report¹ on the action of the vagus in bulbar anaemia induced by temporary occlusion of the head arteries, it was shown that, after division of both vagi, the heart rate does not change during the anaemic rise of blood-pressure, but remains at whatever maximum rate was attained after vagotomy.

It has been thought desirable to find out the effect on heart rate during the cardio-vascular response to bulbar anaemia, of removal of the stellate ganglia through which run the accelerator fibers to the heart. Accordingly, in a series of cats, after a control occlusion had been done according to the usual method,² the stellate ganglia were excised and further occlusions were done. Following is a typical result of such an experiment.

	Heart rate before occlusion	20 secs. after occlusion	130 secs. after occlusion	20 secs. after release
2nd occlusion, control	150	96	236	192
3d occlusion, with stel- lates removed	120	60	180	132

It is evident from the above table, that, with the accelerators eliminated, the vagi are over-active in holding the heart rate down. Under such conditions, recovery of the medulla after the circulation has been restored to the head arteries may, at times, be somewhat difficult, owing to the combination of low blood-pressure and slow heart rate. Moreover, this activity of the vagus is in evidence at a time when blood-pressure is at spinal level and all other signs of bulbar activity are in abeyance. In the experiment which has been given above, the vagi were sectioned in the recovery period, and heart rate increased immediately from 94, at which it had been for about five minutes, to 156; while blood-pressure rose from 40 to 70 millimeters of mercury.

¹ Coombs, *Am. J. Physiol.*, 1924, lxxviii, 124.

² Stewart, *et al.*, *J. Exp. Med.*, 1906, viii, 289.

With both vagi and stellates divided, the heart rate during the remaining occlusion period in the above experiment, remained between 156 and 162, which one would consider relatively constant. Whether the adrenals play any part in the maintenance of heart rate in the denervated heart under conditions of bulbar anaemia, is, at present, under investigation.

210 (2733)

Effect of dyes on the penetration of arsenic into the central nervous system and the spinal fluid.

By C. N. MYERS.

[*From the Department of Dermatology and Syphilology, College of Physicians and Surgeons, Columbia University, New York City.*]

The study of the relation between the antiseptic action and the chemical constitution of synthetic dyes dates back to the period of Paul Ehrlich¹ who began with one group of dyes known as azo dyes, including trypan red, trypan blue and trypan violet. The other group of dyes consisted of basic tryphenyl-methane dyes, including parafuchsin, methyl violet, pyronin G and other similar substances, known as neurotrophic dyes because of their ability to stain nerve tissue.

McIntosh and Fildes² reported that arsenic could not be found in the brain tissue, due to a lack of affinity between the brain substances and the inability on the part of the drugs to penetrate into the brain. *In vitro* experiments show that this is purely a question of physical penetration. The same authors³ classified the dyes as, (1) those which stain the central nervous system, and (2) those which do not stain the central nervous system. They conclude that these variations were dependent upon the

¹ Ehrlich, Paul, and Hata, S., *The Experimental Chemotherapy of Spirilloses*. London: Rebman Limited, 1911.

² McIntosh, James, *The Fixation of Arsenic by the Brain after Intravenous Injections of Salvarsan*, *Proc. Roy. Soc.*, London, 1914, lxxxviii, (B), 320.

³ McIntosh, James, and Fildes, Paul, *Brain*, 1916, xxxix, 478.

question of solubility. The subject further resolved itself into the fact that neurotropic substances are lipotropic, and before a substance can penetrate into a cell it must be soluble in the cell membrane or possess a distinct osmosis.

Kalberlah⁴ decided that there was a distinct increase in the arsenic content when dye was used.

Smith and Waddell⁵ increased the permeability of the choroid plexus to arsphenamine with methyl violet. Their conclusion is that methyl violet when given intravenously does not increase the permeability.

Cornwall and Myers⁶ showed that arsenic actually penetrated the cord and the brain. These experiments are being repeated with the idea of checking up the effect of transfusion just previous to the sacrificing of the animal.

Fordyce and Myers⁷ studied the action of salvarsan, neosalvarsan, silver salvarsan and tryparsamide on penetration of arsenic into the central nervous system in general paresis and cerebrospinal syphilis. A small percentage of patients show no penetration whatever, and a maximum penetration of 192 mg. per 100 gm. of dried specimen has been found. It has been pointed out that there is a significant difference in the chemical physiology of arsenic in the various types of neurosyphilis. The detection of arsenic varies, depending upon whether the lesions are confined essentially to the mesodermal structures or ectodermal structures. The period at which the largest quantity of arsenic appears is likewise dependent upon this type of differentiation. Table I shows the average values for the arsenic present in the brain after intravenous injection of the drugs indicated at the top of the column. It should be noted that the amount of arsenic necessary to produce these values is given at the bottom of the table. Table II shows the composite results obtained with the

⁴ Kalberlah, Fritz, *Muenchen. Med. Wchschr.*, 1922, lxi, 114.

⁵ Smith, Dudley C., and Waddell, J. A., *Am. N. of Syphilis*, 1924, viii, No. 2.

⁶ Cornwall, L. H., and Myers, C. N., *Am. J. Syphilis*, 1923, vii, 287; *Am. J. of Syphilis*, 1923, vii, 629; *Am. J. of Syphilis*, 1924, viii, 726.

⁷ Fordyce, John A., Rosen, I., and Myers, C. N., *Am. J. of Syphilis*, 1922-1925.

TABLE I.

Showing the average values for the brain after intravenous injection of salvarsan, neosalvarsan, silver-salvarsan, tryparsamide, and sulpharsphenamine at the intervals indicated in the left hand column.

Time	Salvarsan	Neo-salvarsan	Silver-salvarsan	Tryp-arsamide	Sulphar-sphenamine
0	39.12	4.53	3.44	2.47	5.76
5 min.	5.51	2.48	0.81	2.43	1.65
10 min.	3.85	0.77	0.66	1.07	1.30
15 min.					0.58
20 min.	1.90	1.80	0.39	1.49	
30 min.	4.90	3.95	1.02	0.17	0.71
40 min.	2.66	0.80	1.43	1.54	
60 min.	6.03	0.46	0.62	0.33	0.27
90 min.	9.15	2.18	2.55	2.05	1.80
120 min.	8.45	1.05	0.98	0.21	0.18
180 min.	1.22	1.40	0.68	1.35	
240 min.	0.56	0.77	1.04	0.46	0.34
300 min.	7.06	0.95	5.69	0.98	
400 min.	7.26	0.55	0.51		1.86
24 hr.	0.39	2.03	1.27	0.32	1.16
48 hr.	9.16	6.02	1.07		7.48
72 hr.	2.36	1.33	0.69	0.62	0.93
96 hr.	1.80	1.89	0.59		0.28
120 hr.	2.71	0.65	0.14	0.14	3.72
144 hr.	7.64	1.17	0.31		3.09
168 hr.	5.39	0.19	0.50	0.03	
Milligrams of drug injected	160	280	160	600	360
Milligrams of metallic arsenic injected	50	51	32	150	65

TABLE II—Average Arsenic Values.

Dye	Interval	No. of rabbits	Spinal Fluid	Blood	Right Kidney	Left Kidney	Liver	Spleen	Brain	Heart	Lung	Cord	Gall bladder
(Neo salvarsan alone)	24 hrs.	1	0.22	0.39	8.98	11.34	0.14	0.69	0.55	2.60	0.18	9.85
"	48 hrs.	1	Trace	0.08	3.27	7.27	0.47	0.35	0.09	2.68	9.03	0.13	4.30
"	72 hrs.	1	Not sufficient	4.40	1.76	3.79	2.69	0.36	0	1.91	0.85	0.13	12.58
Trypan Red	Immediate	1	16.13	8.06	2.69	6.61	2.32	Lost	0.08	2.62	11.36	0.19	7.12
"	48 hrs.	4	10.75	4.14	4.98	4.37	3.06	1.03	0.10	0.97	3.08	0.10	14.43
"	72 hrs.	3	37.78	0.84	3.99	4.33	8.95	0.53	0.07	0.29	1.82	3.51
Bismark Brown R	Immediate	1	69.75	12.27	6.78	7.48	15.39	0.60	0.17	4.05	10.81	0.06	3.41
"	24 hrs.	1	6.39	1.61	5.63	7.94	0.72	1.29	0	2.10	3.51	0.18	3.77
"	48 hrs.	1	40.90	0.11	6.63	4.94	13.54	0.55	0.03	0.08	4.59	0.45	8.94
"	72 hrs.	1	12.85	0.87	1.85	1.82	3.62	0.39	0.39	0.69	3.84	0	8.78
Pyronine G	24 hrs.	1	8.53	3.28	6.45	0.19	0.08	4.50	5.15	0.34	11.31
Methylene Blue	Immediate	1	15.96	3.06	12.25	16.35	5.36	0	0.09	0.29	1.21	0.09	16.26
"	24 hrs.	1	24.09	2.29	5.37	6.42	0.86	1.08	0.11	0.07	2.85	0.20	3.81
"	48 hrs.	2	12.53	0.73	4.71	1.87	8.72	0.12	0.08	0.40	2.16	0.08	9.18
"	72 hrs.	2	36.11	1.07	3.76	4.35	8.13	0.58	0.15	1.68	1.25	0.46	15.95
Trypan Blue	24 hrs.	1	28.58	2.67	5.06	1.07	4.81	0.63	0.12	0.15	4.80	0.40	18.57
"	48 hrs.	2	23.84	4.15	2.47	10.88	12.15	0.32	0.06	0.12	1.27	0.23	18.54
"	96 hrs.	1	40.00	6.97	5.67	7.69	3.73	0.16	0.05	1.70	0.15	0.15	2.41

use of neosalvarsan and the dyes indicated in column 1. The general conclusion that may be drawn from this table is that the presence of dyes has not had any marked effect upon the penetration of arsenic from neosalvarsan into the brain and cord. On the other hand, the presence of the arsenic in the spinal fluid has been markedly increased. The therapeutic possibilities of a condition of this kind have not yet been satisfactorily worked out.

These investigations are being continued with the idea of showing that there are several factors that must be continuously considered in regard to the application of arsenicals in the treatment of various types of syphilis. In the first place, the *quality* of the arsenic which has penetrated, is more important than the *quantity* of arsenic which has penetrated. The physical condition of the arsenic which has reached the lesion is a factor that must be determined in order to evaluate the proper therapeutic index of a given drug. Furthermore, the antibody formation is a feature which is of equal importance in relation to the various types of treatment which are to be employed.

211 (2734)

The chemical composition of the vitreous humor of animal eyes.

By MARTIN COHEN, JOHN A. KILLIAN and NANNETTE METZGER.

[*From the Departments of Ophthalmology and Laboratories, New York Post-Graduate Medical School and Hospital, New York City.*]

A survey of the literature revealed but incomplete data on the chemical composition of the vitreous humor. It was therefore deemed advisable to determine in this body fluid the concentration of some of the compounds known to occur in the blood and cerebrospinal fluid. This work represents a preliminary step in the study of the humors of normal and pathological human eyes. The use of the microchemical methods has made possible the more extensive analysis of the vitreous humors of individual eyes.

Analyses were made of the vitreous humors of the eyes of oxen, horses and pigs. The eyes were removed, without trauma,

from the animals immediately after slaughter. The aqueous humor was withdrawn by a syringe, and the entire vitreous shelled out through an incision in the sclera of the posterior part of the eyeball. After rupturing the membrane, the vitreous was poured upon a C. S. and S. filter paper No. 595, and covered with a watch glass. The filtrate was taken for analysis. All material analysed was fresh. Not more than 45 minutes elapsed between the death of the animal and the beginning of the analysis. The oxen and pigs were young animals, but the horses ranged from 12 to 20 years. The horses were killed instantaneously by a shot into the brain. All animals were free from disease.

The analytical studies included determinations of total solids, specific gravity, proteins, non-protein nitrogenous compounds, chlorides, sugar, phosphorus, sulphur, potassium, sodium, calcium, lactic acid and the ether soluble fraction. All results are reported in terms of mg. of these components to 100 cc. of the filtrable portion of the vitreous. The figures reported in the table are averages of from 6 to 12 determinations. With the exception of sulphur, phosphorus and the ether extract, all determinations have been made upon the humors of individual eyes. For sulphur, phosphorus and the ether extract the humors of several eyes were combined so that 100 cc. were obtained for each of these analyses.

TABLE—Chemical composition of the vitreous humor of animal eyes.

Constituents	Oxen	Pigs	Horses
Specific gravity	1.004		1.003
Total solids (gm. per 100 cc.)	1.11	1.11	1.01 to 1.12
Total nitrogen	21.5	19.9	17.8 to 35.7
Nonprotein nitrogen	15.7	13.6	14.2 to 32.3
Protein nitrogen	6.9	6.3	3.4 to 3.6
Total protein	39.3	39.3	21.2 to 22.5
Urea nitrogen	9.9	8.8	8.0 to 20.0
Amino acid nitrogen	1.8	2.8	2.1 to 3.0
Uric acid	2.8	0.45	1.0 to 2.0
Creatinine	1.1		
Creatine	1.6		
Sugar (as glucose)	39.	30.	71.
Chlorides (as NaCl)	678.	705.	656.
Total sulphur	4.	4.5	2.
Total phosphorus	2.	3.3	
Sodium	301.	318.	311.
Calcium	7.9	7.9	8.5
Potassium	27.9	35.4	24.5
Total ether extract	10.		
Lactic acid	14.8	17.5	17.0

All figures, except for specific gravity and total solids, are for mg. per 100 cc.

212 (2735)

Experiments with extracts of parathyroid glands.

By N. F. FISHER and E. LARSON. (Introduced by Olaf Bergeim).

[*From the Department of Physiology and Physiological Chemistry,
University of Illinois, College of Medicine, Chicago, Ill.*]

In the light of Collip's report at the meetings of the Federation of Societies for Experimental Biology at Washington, D. C., December 1924, it has been deemed advisable to report our experimental work on the extracts of the parathyroid gland.

The parathyroid glands of the ox were used for all preparations of the extracts. Several methods of preparation were tried with varying degrees of success. Our first preparations were found to contain certain toxic substances which produced sterile abscesses at the sites of subcutaneous injection, similar to those produced by impure insulin extracts. This toxic material was later removed, thereby increasing the potency of the extract. The methods of preparation and purification will be reported later.

Our results are in close agreement with those reported by Collip.¹ At present we have three dogs which have had the thyroid and parathyroid glands removed for periods of 3 to 6 weeks. These dogs showed severe tetany in less than 48 hours, following the removal of the thyro-parathyroid apparatus. The tetany was relieved in 2-4 hours after the injections of the extract, relief depending on the size of the dose and the severity of the attack.

Each of the animals received 1-2 pounds of hamburger steak and ground beef hearts, per day. As soon as an attack of tetany was relieved by an injection of the extract, the animal manifested a great desire for food. It was noted that, after the control of the tetany by the extract, the subsequent attacks were less frequent in spite of the fact that the animals were kept on a meat diet.

The chemical and physical changes produced in the blood of the normal and parathyroidectomized dogs by the injection of the extracts, as reported by Collip, have been confirmed. The most profound changes observed were the increases in viscosity, phosphates, calcium and non-protein nitrogen. The maximal effects were manifested in 4-10 hours.

¹ Collip, J. B., *Am. J. Physiol.*, 1925, lxxii, 182.

The symptoms of atonia, depression, diarrhea and dyspnea are readily induced by large doses of this extract.

213 (2736)

Effect of controlled conditions of illumination upon malignancy of transplantable neoplasm.

By LOUISE PEARCE and C. M. Van ALLEN.

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

Periodic variations in the malignancy of a transplantable neoplasm of the rabbit, which have been studied during the last four years, have been correlated with certain meteorological conditions and especially with the actual hours of sunshine. It has been found that periods of maximum and minimum sunlight corresponding with summer and winter were periods of relatively low malignancy; while the periods of greatest malignancy occurred at times of abrupt and rapid changes in the hours of sunshine corresponding roughly with spring and autumn.^{1, 2} The influence of these factors has been interpreted as operating upon or affecting animal economy, while susceptibility or resistance to disease is considered as a function of the animal organism. This conception is supported by analogous variations in experimental syphilis of the rabbit, and the observation that in normal rabbits rhythmic changes in the mass relationship of practically all organs of the body have been found which have a definite relationship to seasonal changes and prevailing meteorological conditions.³ While a variety of factors are probably involved in the production of these fluctuations, there appears to be a close relationship with sunlight and, in particular, first with the amount

¹ Brown, W. H., Pearce, L., and Van Allen, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 371.

² Brown, W. H., Pearce, L., and Van Allen, C. M., *Tr. A. Am. Phys.*, 1924, xxxix, 466.

³ Brown, W. H., Pearce, L., and Van Allen, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 373.

of sunlight as represented by the actual hours of sunshine and, second, with the rate and extent of change in the curve of sunshine. Furthermore, it is of considerable interest that these biological variations take place in the usual animal room with its illumination determined, in the first instance, by prevailing weather conditions and, in the second, by the interposition of window glass through which passes all or most of the sunlight.

In order to test this conception of the relationship between sunlight, and the physical state and functional activities of the animal, experiments were undertaken in which conditions of illumination could be controlled. The particular points studied in our first experiments were the effects induced by a constant illumination of the maximum intensity practicable with simple equipment, and a constant absence of illumination for stated periods of time upon the normal rabbit and upon the course of the disease produced by the malignant tumor. These conditions were controlled in each instance by parallel series of rabbits kept in an ordinary animal room. The investigation has yielded some striking results, and those obtained in the case of the tumor are briefly summarized in the present report.

Experimental: A constant source of light was furnished by twelve 1,000 watt Mazda lamps and three Cooper-Hewitt 50 inch low pressure mercury arcs, Type P, in crown glass, installed in a room measuring 19 x 9.5 x 10.25 feet or 1850 cubic feet, and from which all other light was excluded. It was realized that the quantity and quality of light employed differed in many respects from the diffused light of the laboratory. However, our main object was the maintenance of a constant source of maximum illumination together with the exclusion of the shorter ultra-violet rays such as are filtered out by ordinary glass. The spectrogram of the Cooper-Hewitt mercury arc shows that the crown glass absorbs all light below a wave length of 3022-28 Augström units, while the light of the Mazda lamps is cut off at about 3,100 Augström units. Three superimposed horizontal rows, 2½ feet apart, each consisting of four Mazda lamps and one mercury arc, were arranged in the center of the room, 3¼ feet equidistant from the animal cages placed on either side. It was determined that the intensity of light reaching the cages averaged 425 foot-candles. Ventilation and the maintenance of a satisfactory temperature level were obtained by an electric fan intake of outside air.

A second room was arranged so that all light was constantly excluded except during the time the animals were fed or examined, when a single 30 watt Mazda lamp was employed. This light was not used for more than one hour per day. A third group of rabbits, as controls, was kept in an unaltered animal room which has a southern exposure and is lighted by two large windows. Recording thermometers were installed in each of the three rooms, and recording barometers in the light and control rooms.

The animals employed were male rabbits approximately one year old, and were carefully matched as to breed. They were kept in individual cages and fed the standard diet of oats, hay, and cabbage. Ten rabbits were placed in each of the three rooms on January 16, 1925 where they remained for the duration of the experiment. On February 16th, one month after they had been under the condition of constant illumination, constant darkness, or the variable diffuse sunlight of the laboratory, each rabbit was inoculated in the right testicle with the tumor. The experiment was terminated on April 15th, two months after inoculation, at which time all surviving animals were killed by an injection of air into the marginal ear vein. Detailed records of frequent clinical examinations were kept, and a complete post-mortem examination was made in each instance, special attention being directed to the presence or absence of metastases, the number and distribution of metastases together with an estimation of organ involvement and the state of the growth.

Results: The results of the experiment have been analyzed, as described in previous papers,^{4, 5} upon a basis of (1) the general course of the disease including the type of growth of the primary tumor, the appearance of metastases detected clinically, the physical state of the animal and the mortality rate, and (2) the incidence of metastases and the number, distribution, and state of these growths as found at autopsy. In this report, certain of the outstanding differences in the behavior of the tumor in the three groups of animals will be briefly summarized.

The malignancy of the disease in the control group was on a comparatively high level. The primary tumor grew rapidly and extensively during the first three weeks, after which time regres-

⁴ Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1923, xxxviii, 347.

⁵ Brown, W. H., Pearce, L., and Van Allen, C. M., *J. Exp. Med.*, 1924, xl, 583.

sion took place in the 5 rabbits which survived to the end of the experiment, and in 3 of these complete absorption with healing occurred; in the 4 rabbits dying from the tumor, the primary growth was largely living. Ten superficial metastases were detected clinically in 4 animals. There was one death from an intercurrent infection, but the mortality rate for the series due to the tumor was 44 per cent, 4 rabbits succumbing four to five weeks after inoculation. The animal incidence of metastases as found at autopsy was 56 per cent, and there was a total of 73 metastatic foci in the entire group distributed as follows: 27, 15, 14, 13, and 4; while no metastases were found in four rabbits.

A definitely less malignant disease developed in the rabbits in the dark room. The group almost immediately became divided in two parts: In 3 rabbits the primary tumor grew very rapidly, and these were the animals which succumbed to the disease $3\frac{1}{2}$ and 4 weeks after inoculation. In 7 animals the primary tumor grew more slowly, and ultimately regressed. In 4 rabbits, 7 metastases were found clinically, while the mortality rate of the group was 30 per cent. The animal incidence of metastases was 40 per cent, with a total of 65 foci of secondary growths distributed as follows: 25, 23, 16, 1; and none in six rabbits.

In the light room, however, the malignancy of the disease dropped to a very low level. The initial growth of the primary tumor in the majority of the animals was unusually rapid and extensive, but in 9 rabbits it eventually became entirely necrotic or was completely healed. In addition, 6 metastases were found clinically in only 2 animals. In one of them the metastatic growth in the iris of both eyes became completely healed—an unusual occurrence; while in the second rabbit there were 4 superficial lymph node metastases which developed later than is ordinarily seen, and $6\frac{1}{2}$ weeks after inoculation the animal was killed because of a slowly developing cachexia, partial paralysis of the hind quarters, and the occurrence of convulsions. The mortality rate was only 10 per cent, as contrasted with 30 per cent in the dark and 56 per cent in the control groups. Postmortem examination showed that in 5 animals there were 24 foci of metastases distributed as follows: 19, 2, 3, with 1 each, and none in 5 rabbits, but in the animal with two, and in one of those with one metastasis, these growths were small and entirely necrotic. It is certain that none of the surviving 9 rabbits would have died from the effects of the tumor.

Conclusions: It is evident that under the conditions of the experiment the establishment of practically constant darkness produced a slight but definite influence upon the course and malignancy of this tumor so that those animals which possessed a high resistance to the tumor, either natural or acquired, were enabled to exercise this effect more efficaciously. But such an influence was not sufficient to enable less resistant animals to cope successfully with the disease. On the other hand, with the conditions of constant light, even without the short ultra-violet rays to which marked physiological effects have been ascribed, the disease assumed an extremely mild form—only one animal in the group dying from its effects, and this occurred later than the 3 deaths in the dark group or the 4 among the control rabbits. It would appear, therefore, that in this environment of artificial light a restraining or inhibitory action upon this disease was developed.

The results of this experiment furnish confirmatory evidence to the conception already referred to that there is a correlation or relationship between the light environment surrounding the rabbit and the course and malignancy of this neoplasm. We interpret these effects of controlled conditions of illumination as being developed and exercised through the operation of the factors of resistance of the host which were evidently more potent or efficacious than was the case under the controlled conditions of the diffuse sunlight of the laboratory.

Finally, it may be mentioned that no demonstrable ill effects of a general nature developed in the tumor animals of this experiment or in a large number of normal rabbits caged in the same rooms. Very definite changes, however, occurred in the mass relationships of various organs of the normal rabbit, and these will be presented in a subsequent communication.

214 (2737)

Studies on the ultrafiltration and electrodialysis of insulin solutions.

By T. C. TAYLOR, C. E. BRAUN and E. L. SCOTT.

[From the Division of Chemistry and the Department of Physiology, Columbia University, New York City.]

Insulin (Lilly) was ultrafiltered through graded collodion membranes, and also subjected to electrophoresis and dialysis combined. No apparent purification was obtained by the first method, but by the second a definite fractionation into protein-like materials was accomplished. One of these fractions was very active, while the other showed very little activity. Each contained about 14.5% of nitrogen; and the active portion contained sulphur, while the inactive portion did not. The isoelectric point of the inactive material was pH 4.8 to 5.0, which is the same as that reported for the original material; while that of the active portion is apparently 5.0 to 5.2.

At pH of 3.6 all of the active material deposits on the negative membrane during the passage of the electric current, while the inactive material remains in solution. If the current is stopped there is immediate resolution, showing that the deposit is not an isoelectric precipitation. Adjustment of a solution of this material to a pH of 5.0 causes a precipitation. The ash from this material constituted about 2% of the total dry weight, and consisted only of silica. Repeated treatments did not remove this ash. The physiological activity of the active fraction is about 100% greater than that of the original insulin from which it was prepared, per unit of dry material.

Treatment of the active sediment with 25% sulphuric acid to which an equal volume of alcohol has been added causes a complete solution. On long standing of this solution, at room temperature, small needle crystals deposit. These crystals continue to form until all of the solid material originally dissolved appears in micro-crystalline form.

SUMMARY

The procedure described gives a method for further concentrating the active principle of insulin without the use of added

reagents, precipitants, adsorbants or whatnot. It accomplishes a separation of insulin, as prepared commercially, into active and inactive protein-like substances the isoelectric points of which are so close together that fractional precipitation as ordinarily practised is almost impossible.

Crystals, the chemical and physiological properties of which are being investigated, are formed from the active sediment, either by cision or by compound formation.

215 (2738)

The Ramon flocculation test in relation to the antigenic value of diphtheria toxoid (anatoxin).

By ABRAHAM ZINGHER.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

In a series of publications, Ramon¹ and Glenny, Hopkins and Pope² have claimed that the antigenic value of diphtheria toxoid (anatoxin) can be determined by the flocculation test described by Ramon. Since anatoxin, as defined by Ramon, is completely atoxic, the L+ and Lo cannot be used for determining the neutralizing and antigenic values of such a preparation. There are three other tests, however, that can be utilized:

1. The flocculation reaction of Ramon;
2. The combining value for antitoxin, as shown by the neutralization test;
3. The immunizing value in guinea pigs.

The flocculation reaction of Ramon, as will be shown below, is not a true index of the antigenic value of toxoid (anatoxin). Baecher, Kraus and Lowenstein³ have also come to the same conclusion as a result of their work with guinea pigs.

In a recent communication Nelis⁴ states that while anatoxin

¹ Ramon, G., *Ann. de l' Inst. Pasteur*, 1925, xxxix, 1-21.

² Glenny, A. T., Hopkins, B. E., and Pope, C. G., *J. Path. and Bact.*, 1924, xxvii, 261.

³ Baecher, S., Kraus, R., and Lowenstein, E., *Zeitschr f. Immunitats f.*, 1925, xlii, 350.

⁴ Nelis, P., *C. R. de la Soc. de Biol.*, 1925, xcii, 1112.

preparations, which contain the largest amount of toxoid, have the best antigenic value, yet this property does not depend exclusively upon its presence in the anatoxin. He found that toxins inactivated by different agencies and in which toxoid could not be demonstrated any longer by its neutralizing power for antitoxin were still capable of producing immunity, although the appearance of the immunity was somewhat retarded. Such agencies were: prolonged exposure to incubator temperature, action of ozone on toxin, and toxins inactivated by sodium oleate (0.04 per cent) or by quinine bichlorhydrate (0.01 gm. to 50 M. L. D. of toxin).

In this connection it is of interest to note that Bronfenbrenner and Reichert⁵ working with *Botulinus* toxin found that "a young toxin, though it be physiologically more potent than an older one and productive in animals of a highly antitoxic serum, may not produce precipitins; whereas a filtrate from a culture old enough to contain presumably a relatively high concentration of bacterial protein in addition to toxin produces a flocculating as well as antitoxic serum when used for immunization of animals." They conclude that the use of the flocculation reaction for the *in vitro* titration of *Botulinus* antitoxin is limited by the fact that the flocculating power is not strictly parallel to toxicity, but depends upon the presence of bacterial proteins in the antigen.

The combining value for antitoxin is shown by the addition of an excess of antitoxin and then by titrating the unneutralized antitoxin by toxin. According to Glenny, Pope and Waddington,⁶ this method has one drawback: These observers claim that toxin has a tendency to dissociate the antitoxin from the toxoid, and then to combine with the antitoxin. This is due to the greater affinity of antitoxin for toxin than for toxoid.

The third method is by testing the immunizing value of toxoid (anatoxin) in guinea pigs. One human dose is given to a series of 10 or 12 guinea pigs. After four weeks a Schick test is made with 1/50 M. L. D. toxin on the denuded lateral side of the abdomen. Note is taken of the local reaction after 48-72 hours. The positive reactors are retested after 2 weeks on the opposite

⁵ Bronfenbrenner, J., and Reichert, P., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 391.

⁶ Glenny, A. T., Pope, C. G., and Waddington, H., *J Exper. Path. and Bacteriol.*, xxviii, 279.

side of the abdomen. The percentage of negative reactors at each test will serve as a guide of the antigenic value.

The immunizing results in the guinea pigs are determined not only by the skin test with toxin but also by injecting the negatively reacting animals with amounts of toxin varying from 5 to 100 M. L. D., *i. e.*, 5, 10, 25, 50, 75 and 100 M. L. D. Such tests I made recently on 10 guinea pigs injected with 0.5-1 cc. of Ramon's anatoxin. Five of the animals gave a slight positive skin reaction to 1/50 M. L. D. at the end of 6 weeks; the others were negative. A second skin test made 2 weeks later with a similar amount of toxin showed that 9 of the 10 guinea pigs gave negative reactions. These animals were injected with doses of toxin varying from 2.5-100 M. L. D. The guinea pigs receiving 2.5, 5 and 10 M. L. D. showed no local reactions. Those receiving 25 to 50 M. L. D. had local induration and necrosis, but recovered. After 75 M. L. D. the animal survived for 8 days; after 100 M. L. D. the guinea pig died after 48 hours. Even 0.2 cc. of Ramon's anatoxin protected after a period of 6 weeks three guinea pigs against 50 M. L. D. of toxin, although the animals showed considerable local induration at the site of the toxin injection.

THE FLOCCULATION REACTION OF RAMON

This depends upon the addition of graded amounts of anti-toxin to a definite amount of toxin. The tube showing the most complete neutralization will be the first one to show flocculation. The rapidity of the appearance of the reaction depends upon the strength of the toxin, the temperature of the waterbath or incubator, the reaction of medium, etc.

In preparing our toxoid with formalin we noted that the preparation first became opaque and that a precipitate formed after the toxoid remained for a few days in the thermostat. This settled out later in the ice box as a heavy sediment. *This precipitate carried down with it the flocculable substance.* After filtration through a Berkefeld, the clear filtrate tested with the Ramon test showed no flocculation. It had, however, retained all its immunizing value. Such a filtrate gave as good immunity results as the unfiltered toxoid when the amounts injected were equal.

The precipitation in the toxoid was due to the action of the formalin upon the toxin to which 0.4 per cent tricresol (alcrestol)

RAMON TEST AND ANTIGENIC VALUE IN DIPHTHERIA 457

had been added as a preservative. Toxin without tricresol showed no precipitation upon the addition of formalin. The addition of formalin in amounts increasing from 0.1 per cent to 0.75 per cent produced varying degrees of opacity, depending upon the amount of formalin added. When phenol is used as a preservative, the precipitate formed may be very slight or absent. Ramon adds no preservative to the diphtheria toxin, and has never noted such a precipitate.

Immunity Results in School Children Showing the Relation Between the Ramon Flocculation Test and the Antigenic Value of Diphtheria Toxoid (Anatoxin).

Preparation	Berkfeld Filtration after formalin treatment.		L f Per cc. Toxoid	Doses.		Clinical Results.	
	For Flocculation Test	For Active Immunization		Number	Amount cc.	No. Tested	Per Ct Neg. on Schick Retest
1—Toxin 589	No		4 units				
2—Toxoid 589	Yes		0				
3—Toxoid 2 C	Yes	Yes	0	3	0.5	226	79.0-95.0
4—Toxoid 10	Yes	No	0	3	1.0	124	98.0
5—Toxoid 7	Yes	No	0	3	.25	359	58.0-86.0
6—Anatoxin, Ramon	No	No	10	3	0.5-1.0	205	98.0
7—Toxoid 437	No	No	2.5-3.5	3	0.05-0.1	1500	84.0-94.0
8—Toxoid 377	No	No	2.0	3	0.07-0.1	1200	27.0-63.0
			0				

The table shows that Toxin 589 gave a flocculation test with 4 units of antitoxin per cc. After the addition of formalin (0.25 per cent) the typical cloudiness and precipitate developed. Berkfeld filtration cleared the toxin. No flocculation occurred when antitoxin was now added in amounts varying from 12 to 1 units to one cc. of toxoid. This preparation (Toxoid 589) was not used for human immunization.

Toxoid 2 C was similarly treated with formalin and cleared by Berkfeld filtration. *No flocculation was noted with the cleared filtrate. Three doses of 0.5 cc. each immunized from 79 to 95 per cent of children.*

Toxoid 10 was passed through the Berkfeld filter for the flocculation test, but used for human immunization without filtration. No flocculation was noted. Of the injected children, 96 per cent became immune with 3 doses, each 1 cc.

Toxoid 7 was not passed through the Berkfeld filter for the flocculation test. No flocculation was noted. For immunization the cloudy preparation was used. Three doses of 0.25 cc. only immunized from 58 to 86 per cent of the injected children.

Anatoxin (Ramon) consists of several preparations sent to me by Ramon. No Berkfeld filtration was necessary as the preparation was clear. The flocculation test showed that 10 units of antitoxin gave the initial flocculation with 1 cc. of the anatoxin. Injected into guinea pigs in doses of 0.5 to 1.0 cc., 90 per cent of the animals gave a negative skin reaction to 1/50 M. L. D. of toxin after 8 weeks, a preliminary test having been made 2 weeks previously.

Among children, 98 per cent gave a negative Schick test 6 weeks after the third dose of anatoxin. The doses were 0.5 cc., 0.5 cc., and 1.0 cc., given at intervals of two weeks.

The flocculation and animal immunization tests on Toxoids 437 and 377 were kindly carried out for me 6 months ago by Ramon, by Glenny of the Burroughs, Wellcome & Co. Laboratories, and by Moloney of the University of Toronto. These observers obtained somewhat different results. With Toxoid 437 the Lf value was found to vary between 2.5-3.5 units per cc. A recent test showed that the toxoid had lost its power to flocculate with antitoxin, although the immunizing value for guinea pigs was not impaired.

Toxoid 377, according to Moloney, had a flocculating value of

20; according to Glenny of 2; according to Ramon 0.5 unit of antitoxin only produced a cloudiness after 24 hours with 1 cc. of toxoid.

In guinea pigs, according to Banzhaf, Toxoid 437 in 0.1 cc. dose immunized (negative Schick test) only 15 per cent of the animals, and Toxoid 377 in 0.1 cc. dose 50 per cent of the animals. According to Moloney 0.2 cc. of Toxoid 437 immunized 2 out of 3 guinea pigs and 0.2 cc. of Toxoid 377, all of 3 guinea pigs; he concludes, however, "that the immunizing power of the two toxoids for guinea pigs is about the same." According to Glenny, 1 cc. of Toxoid 437 immunized guinea pigs in 15 to 18 days; 1 cc. of Toxoid 377 in 19 to 21 days. The first (437) required 2 to 3 previous Schick tests. According to the animal results Banzhaf found Toxoid 377 about 3 times as efficient as Toxoid 437; Moloney found them of equal value and Glenny found Toxoid 437 to be more efficient than Toxoid 377.

In the active immunization of children I obtained strikingly different results, using the two toxoid preparations in doses of 0.05-0.1 cc. Of 1500 children injected in different schools with three doses of Toxoid 437, from 84 to 94 per cent gave a negative Schick retest; of 1200 children injected with Toxoid 377, 27 to 63 per cent gave a negative Schick retest.

CONCLUSION.

The flocculation reaction does not appear to be an index of the antigenic value of a diphtheria toxoid (anatoxin).

The reaction is probably a specific bacterial precipitation phenomenon.

216 (2739)

Early and late immunity results with scarlatinal streptococcus toxin.

By ABRAHAM ZINGHER.

[From the Bureau of Laboratories, Department of Health, New York City.]

From the results noted with the Dick test¹ among large numbers of persons of different age and social groups, it was evident that a close analogy would be found not only in the natural antitoxic immunity but also in the artificially induced antitoxic immunity to scarlet fever as well as to diphtheria. The results with the Dick re-test on over 1,200 individuals (originally positive) injected in schools, institutions and private practice, justify the conclusion that an active immunity can not only be developed in most persons, but that it is persistent in 80-90 per cent of those successfully immunized. About 10 to 20 per cent of the injected may show a slightly positive Dick reaction after having had a negative reaction at a previous test. The antitoxic immunity in these persons can be quickly re-established with a few doses of toxin. My own three children, whose ages are two, three and one-half and five years, and who were injected 14 months ago with three doses of the toxin, still give a negative Dick retest.

Our standard solution of toxin for the Dick test is just twice as strong as that recommended by the Dicks. It indicates better the susceptible group of children by their more pronounced positive reactions. Slight positive reactors are not considered as being very susceptible to the toxic phase of scarlet fever. The stronger toxin dilution also indicates that the person injected with it and giving a negative Dick reaction at the retest is more certain to be immune to scarlet fever. It also shows in the naturally immune a larger amount of antitoxic antibodies and therefore these individuals are less likely to give positive reactions at subsequent tests. A method of standardization of the toxin for the Dick test was suggested by Zingher² in a previous communication.

The work now covers a period of fifteen months. We used small doses at first to avoid the severe local and constitutional

¹ Zingher, Abraham, *J. Am. Med. Assn.*, 1924, lxxxiii, 432.

² Zingher, Abraham, The Dick Test and Active Immunization with Scarlet Fever Streptococcus Toxin. *Am. J. Public Health*, March, 1924.

reactions described by the Dicks in their original communication on active immunization with the scarlatinal toxin. With increasing experiment we have gained more confidence in the use of the toxin, and have not hesitated to increase the dosage from the original three doses recommended, 100, 250, and 500 skin test doses, to four doses of 250, 1000, 2000 and 3000 or 4000, depending on the age and reactions of the injected person.

The study of the immunity response has been very interesting. Just as in the active immunization against diphtheria, we find in the immunization against scarlet fever also, that those who have a slight amount of antitoxin and show a \pm reaction give a better and prompter response than those with a + or ++ reaction. But even most of the ++ reactors can be fully immunized with one or two series of toxin injections. Since using the larger doses of toxin, we have obtained with one series of injections about 70 per cent negative Dick re-tests, and about 26 per cent of slightly positive re-tests among persons who originally gave ++ or + reactions. We have not found that the addition of phenol as a preservative to the toxin interfered in the slightest degree with the immunizing value of the toxin.

In two private schools, the Ethical Culture School and the Horace Mann School, not a single case of scarlet fever developed among the injected children, although outbreaks of scarlet fever developed in both schools 3 to 9 months afterwards. At the Ethical Culture School about 200 out of 800 children, and at the Horace Mann School about 250 out of 900 children, have been injected.

It is interesting to note that the negative Dick reaction in "naturally" immune persons is quite permanent. Of 2,580 such negative reactors re-tested after 2 to 12 months, 2,545 of 98.6 per cent, continued to show a negative Dick reaction. This indicates that in the persistence of the natural immunity to scarlet fever we have a close similarity to that noted to diphtheria. With the Schick test we had seen on re-testing over 12,000 naturally immune school children after a period of 6 months to 3 years, that only 11 children (or less than 0.1 per cent) subsequently showed a positive Schick reaction.

CONCLUSION.

1. A complete antitoxic immunity to scarlet fever was produced by 4 doses of scarlet fever toxin (250, 1000, 2000 and 3000 skin test doses) in 70 per cent of injected persons, and a

partial immunity in 26 per cent. A second series of toxin injections successfully immunized most of the refractory cases.

2. Subsequent exposure to scarlet fever showed that the induced active immunity protected the children from infection.

3. From 10 to 20 per cent of individuals who gave a negative Dick reaction after toxin injections showed a slightly positive reaction at later re-tests.

4. Groups of children who were re-tested from 9 to 14 months after the toxin injections show by a negative Dick retest that the immunity can last for at least that period of time.

217 (2740)

Immunity results with diphtheria toxoid (modified toxin anatoxin) and 1/10 L + mixtures of toxin antitoxin.

By ABRAHAM ZINGHER.

[From the Bureau of Laboratories, Department of Health, New York City.]

The 1/10 L+ mixture of diphtheria toxin-antitoxin, when prepared with a slight excess of free toxin so that 5 cc. will kill a guinea pig in 5 to 7 days, is an efficient immunizing agent. Three doses, each 1 cc., will immunize from 90 to 98 per cent of injected persons. The fact, however, that an underneutralized mixture has to be used which produces the local effects of free toxin at the site of injection; that the mixture has to be very carefully prepared so that the amount of free or partly bound toxin is not in excess; and finally that the mixture on standing loses some of its immunizing value, indicates that such a mixture is not yet the ideal preparation for the universal immunization of all children of school age and pre-school age. In diphtheria toxoid (anatoxin) we have such a preparation. It is easily made by adding 0.25 to 0.4 per cent formalin to the toxin, and leaving the formalinized toxin in the incubator for 30 days. The preparation is stable, *non-toxic* and highly efficient as an immunizing agent. With a preparation sent us by Ramon, we injected one-half of the children in three large schools, the other half being injected with 1/10 L+ toxin-antitoxin. The dose of the anatoxin

was as follows: 1st dose, 0.5 cc.; 2nd dose, 0.5 cc.; and the 3rd dose, 1.0 cc. The dose of the toxin-antitoxin was 1 cc., repeated three times. The injections were given every two weeks. The Schick re-test was made in one of the schools at the end of 6 weeks, and the following results were noted:

- (A) Three doses anatoxin 98.0% Negative Schick re-test.
- (B) Two doses anatoxin 86.4% Negative Schick re-test.
- (C) One dose anatoxin 60.0% Negative Schick re-test.

With the toxin-antitoxin, 91 per cent of all the injected children gave a negative Schick re-test. The local reactions to the toxoid (anatoxin) were slight. In fact the toxin-antitoxin, being slightly toxic, gave more pronounced local reactions. In pseudo-reactors the toxoid (anatoxin), being undiluted formalinized toxin, gave more marked local reactions.

The diphtheria toxoid prepared at the Research Laboratory was slightly toxic, following the suggestions of Glenny, Hopkins and Pope. On account of the excess of free toxin we^{1, 2} used only small doses of the toxoid at first (0.1 to 0.05 cc.). From 52.0 to 94.0 per cent of over 3,000 school children injected with these doses in different schools gave a negative Schick re-test. By increasing the dose with later preparations, the results were better and more uniform.

With one preparation of toxoid after three doses, each 1 cc., 96 per cent of the children showed a negative Schick re-test; with another toxoid after three doses, each 0.5 cc., 79 to 95 per cent became immune.

The smaller the amount of formalin used, the better were the immunity results. From one toxin, three preparations of toxoid were made—one with 0.25 per cent formalin (Toxoid 7), one with 0.5 per cent (Toxoid 7 A), and one with 0.75 per cent (Toxoid 7B). Three doses of 0.25 cc. each were given of the 0.25 (Toxoid 7) and of the 0.5 per cent formalinized toxin (Toxoid 7 A), and three doses of 0.5 cc. each of 0.75 per cent formalinized toxin (Toxoid 7 B). Of 359 children receiving Toxoid 7 (0.25 per cent formalin) 66 per cent gave a negative Schick re-test after 3.5 months; of 336 children receiving Toxoid 7 A (0.5 per cent formalin) 45.8 per cent were negative at the re-test,

¹ Zingher, A., and Park, W. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1924, *xxi*, 383.

² Park, W. H., and Zingher, A., *Am. J. Dis. Child.*, 1924, *xxviii*, 464.

and of 100 children receiving Toxoid 7 B (0.75 per cent formalin) 36 per cent were negative.

CONCLUSION.

Diphtheria toxoid (anatoxin) is an ideal preparation for active immunization against diphtheria. This is especially true for children of school age and of pre-school age who are relatively free of protein sensitiveness and in whom the toxoid produces very slight local or constitutional reactions.

218 (2741)

Studies upon the biological reactions of growing tissues to radiant energy. I. Effect of radiumized media upon tissue cultures *in vitro*.

By MAURICE J. SITTENFIELD and BALBINA A. JOHNSON.

[From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.]

The *in vitro* cultivation of embryonic tissues seemed to us the most promising method of gaining important information of the biological reaction of growing tissues to radiant energy. Up to the present, experimentation along physical lines has claimed major interest, and very scant attention has been paid to the biological importance of radiant energy. Consequently very little is known concerning the biological phenomena accompanying radiation.

It was highly desirable therefore to study the immediate and latent behavior of tissues growing *in vitro*, when placed in immediate contact with radium salts and radium emanations.

The experiments to be reported were conducted upon chick embryo hearts, embryo spleens, and upon Flexner-Jobling rat carcinoma. Along with these, numerous experiments were made upon the effects of radium salts, radium emanations, as well as X-rays, upon different media, such as plasma, serum, embryonic tissue extracts, etc.

In the present studies chick embryo hearts from 7 to 11 day embryos, and spleen from 18 to 20 day embryos were used for *in vitro* studies. Small fragments were planted in a medium of chicken plasma and embryonic tissue juice. In the cultures where

EFFECT OF RADIUMIZED MEDIA UPON TISSUE CULTURES 465

spleen tissue was used, the embryonic tissue extract was omitted. Fine glass seeds about 0.5 centimeter in length, and 1 millimeter in thickness, containing 0.1, 0.25, 0.35, 0.5 millicuries to 1.0 millicurie of radium emanations, were placed in direct contact with the tissue, or, 1 and 2 millimeters distant.

A series of 12 experiments were conducted comprising 100 cultures, without radium or with empty glass seeds. Growth was charted by using the camera lucida, and the area of new growth measured with the planimeter one hour, 24 hours, and 48 hours after incubation. After this period subculturing was continued.

In the 133 controls a normal growth was observed in 124 cultures, 9 cultures showed either no growth or a very scanty one.

In the 100 cultures treated with radium emanation 49 showed no growth whatsoever, 30 were markedly inhibited, especially on the side toward the radium seed, and in 21 growth was fair or normal. It was of especial interest to observe that cultures with 0.1 millicurie of radium emanations were effected in practically the same proportion and manner as those with the larger amounts.

Aside from these experiments with radium emanation, other cultures were treated by applying needles containing 5, 10, 25, and 50 milligrams of Radium Bromide to the mica cover slip over the small fragment of tissue, for varying lengths of time. In the majority of these cultures growth occurred, but the outgrowth was studded with fine vacuoles indicating early degeneration, and upon subculture, showed complete degeneration within 8 to 10 days.

Experimental						Control		
Growth 48 hours						Growth 48 hours		
No. Exp.	No. Cult.	Mc. Rad.	No Growth	Inhibited	Fair	No.	No Growth	Normal
18	8	0.1	0	8	0	8	0	8
22	6	0.13	5	1	0	6	0	6
38	10	0.24	3	4	3	10	0	10
50	4	1.05	4	0	0	19	0	19
54	10	0.25	7	3	0	19	5	14
64	11	0.12	5	5	1	35	2	33
70	6	0.5	0	3	3	9	0	9
72	18	0.25	12	0	6	9	0	9
74	12	0.25	5	6	1	10	1	9
80	5	0.35	4	0	1	4	0	4
92	5	1.02	0	0	5	5	1	4
93	5	0.21	4	0	1	4	0	4
Totals	100		49	30	21	138	9	129

In connection with this work I wish to express my appreciation to Dr. Muir, who supplied the Radium Emanation seeds.

219 (2742)

A biochemical reaction associated with sex in Cladocera.

By ARTHUR M. BANTA and SOPHIA SATINA.¹

[*From the Station for Experimental Evolution, Cold Spring Harbor, L. I.*]

A biochemical test for sexual differences reported by Manoilov² and used as the Station for Experimental Evolution by Miss Sophia Satina and others with various plants and animals, has been applied to three races of Cladocera. This is probably the first time that such a biochemical difference between the sexes has been demonstrated in a crustacean.³

The first test with Cladocera was with small samples (0.02 gram) of females and males of *Moina macrocopa*. The female sample gave a faint violet coloration. The male sample gave no color. These samples, though each involving 200 or 300 individuals of this minute species, were too small to give the striking reaction obtained with more material.

The other Cladocera used were obtained from the wilds near Cold Spring Harbor. One form, a distinct type closely related to *Daphnia pulex*, was in an actively sexual condition. Every female was producing sexual eggs, and adult males were in the population in great numbers. A sample in duplicate of the females consisting of nearly 200 individuals was taken. A second sample was of males exclusively, about 350 individuals. A third sample was of a different type of *Daphnia pulex*, but obtained at the same time and from the same pond. The third sample was of a stock which was exclusively in parthenogenetic reproduction,

¹ Research carried on with the aid of a grant from the Committee for Research in Problems of Sex of the National Research Council.

² Manoilov, E. O., *Bul. Appl. Bot. and Plantbreed*, 1922-1923, xiii (2), 503.

³ A statement of the method used is appearing in *Science*, "Manoilov's Reaction for Identification of the Sexes" by Sophia Satina and M. Demerec.

each individual bearing numerous eggs, embryos or young in the brood chamber. The samples were of like size,—weighing 0.13 grams after the water had been removed as far as possible with filter paper. The animals were then placed in like amounts of 60 per cent alcohol (2 cc.) and the reagents applied. The sexual female sample developed a pronounced violet color. The parthenogenetic females showed the same color, but with much less intensity. But the males showed none of the coloration.

These results taken in connection with numerous tests made by Miss Satina on plants and the blood of certain vertebrates, show that these invertebrates give a biochemical reaction for sex comparable with that given by plants and higher animals.

220 (2743)

Studies of the formation of the streptococcus toxin.

By F. KRASNOW, R. G. FREEMAN, JR., and E. G. MILLER, JR.

(Introduced by William H. Park).

[From the Laboratory of Biological Chemistry of Columbia University at the College of Physicians and Surgeons and the Board of Health Laboratory, New York City.]

In a paper read before the American Society of Bacteriologists in December, 1924, Huntoon reported observations on the progressive formation of skin-reacting toxin in the Berkefeld filtrate from a 44 hr. culture of hemolytic streptococcus (scarlet fever). He suggested that this formation of toxin in the absence of organisms was probably due to the action of an enzyme (protease), secreted by the bacteria, in the filtrate. The following experiments were conducted to determine something of the nature of this enzyme action. The organism used was a strain of hemolytic streptococcus isolated from a scarlet fever patient and obtained from the Board of Health.

Qualitative tests on filtrates obtained by passing a 36 hr. culture (bacto-veal-horse-plasma broth) through a Berkefeld filter, and on extracts of the bacterial bodies (obtained as described below), failed to show the presence of active protease, using

as substrate either fibrin or a solution of proteose (prepared from bacto-peptone).

Mass cultures were made by growing the streptococcus on whole blood bacto-veal agar. The growth was carefully removed and dried over concentrated sulphuric acid. Three tenths gm. of dried bacteria was well ground and suspended in a M/15 Na_2HPO_4 , KH_2PO_4 mixture having a pH of 7.2. This was shaken at intervals during 12 hours, and filtered through Berkefeld. This filtrate was designated "bacterial extract."

Cutaneous tests were made by intradermal injections, using the Dick technique, in the arm of an adult male subject who was skin-sensitive to the Dick toxin. After many repeated injections in this subject, it seemed that some change occurred in the skin which made the readings of the tests difficult to interpret. The later experiments, therefore, were done on infant subjects who gave positive (4 plus) reactions to the Dick test, and negative controls. The skin reactions in the various series were checked by neutralization tests with known convalescent scarlet fever sera.

Simultaneous series were run on blood broth alone, bacterial extract alone, and broth and extract mixed; unincubated, and incubated at 37°C . for 1 to 8 days; and in varying dilutions. Also, some series were done on bacterial extract incubated with solutions of serum albumin and globulin.

The results may be summarized as follows: The extract alone gave a skin reaction only in low dilution; on incubation there was a slight but progressive increase, generally to about the eighth day. Blood broth alone gave no reaction. The extract and broth together showed a progressive increase in reaction which was definitely and consistently, though slightly, greater than in the extract alone. Incubation of the extract with serum proteins gave negative results. In none of the series was the increase obtained by us comparable in magnitude to that reported by Huntoon.

It would seem that, if the increase of skin-reacting toxin in the absence of the organism is due to enzyme action, this enzyme is rather an exo-enzyme, which is not stored to any great extent in the bacteria. The increase of toxin by incubation of the extract alone, on the enzyme hypothesis, may be considered as perhaps due to protease action on small amounts of bacterial protein in

the extract. The negative results obtained with serum protein suggest either that these proteins may not be the substrate from which toxin is formed, or that some "x factor," in addition, is needed.

221 (2744)

The effects of radiation on calcium and phosphorus.*

By H. S. MAYERSON, LEWIS GUNTHER and HENRY LAURENS.

[From the Department of Physiology, Yale University, New Haven, Conn.]

As a part of a problem on the effects of darkness and of radiation on the metabolism of normal dogs, we followed the balances and blood levels of calcium and phosphorus before and after radiation with a 25 ampere flaming arc, with a spectral energy distribution of approximately 50 per cent ultra violet, 11 per cent visible, and 39 per cent infra red. The animals were fed on a standard maintenance diet furnishing 70 calories per kilo body weight, and well balanced and complete in calcium and phosphorus. All the animals were on positive balances at the beginning of the experiments.

Under normal laboratory conditions there is a balance between the two constituents, a slight rise in serum phosphorus being accompanied by a similar decrease in the serum calcium, and vice versa. Radiation of one hour at 40 cms. (total energy equivalent to 55.44 gm. cal. per cm.²) for 8 days served to accentuate this balance, there being a marked increase in the phosphorus and a corresponding decrease in the calcium during the radiation, and a return to normal soon after the radiation was stopped. On repeated exposures of the same duration, however, both constituents show almost parallel curves, a rise in phosphorus being accompanied by a simultaneous increase in calcium. Single doses of two hours for 8 days on other dogs gave results similar to those obtained on repeated exposures. Grant and Gates¹ have reported similar findings in the rabbit.

* Aided by a grant from the Elizabeth Thompson Science Fund.

¹ Grant and Gates, PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 315.

Examination of the urine and feces shows that, although in some cases the actual amount of excretion is not markedly increased, the percentage found in the urine relative to the feces is always larger, indicating an increased absorption and retention of these constituents in the blood. Orr, Holt, Wilkins and Boone² and others have found similar effects on radiation in rickets and on diets deficient in calcium. Our results seem all the more significant since they were obtained on normal animals on complete diets, and suggest the possibility of stimulating balances, already positive, to optimum by radiation. In order to be able to make accurate comparisons, we have measured the radiation used spectrographically and spectroradiometrically, and express the dosage in absolute units.

222 (2745)

A brief note on the anatomy of the uterine opening of the Fallopian tube.

By FERDINAND C. LEE.

[*From the Anatomical Laboratory of the Johns Hopkins University, Baltimore, Md.*]

A brief preliminary report¹ was recently made to call attention to the fact that in some of the laboratory animals it was always easy to inject from tube to uterus, whereas, when an injection was made in the reverse direction, the injection mass would pass more readily when the animal was found to be near the time of ovulation. Serial sections were made of the uterine opening of the Fallopian tube in the cat, dog, rabbit, rat, guinea pig, and pig. In all cases the opening of the tube was guarded by special folds, which, in the case of the pig, were easily visible in the gross after fixation. A single human specimen failed to show the folds. These special folds were free of glands in the case of the cat, rat, and pig; whereas in the dog a few glands were found. Furthermore, the stroma in these folds contained a large amount of connective tissue so that these structures were not flattened to the

² Orr, Holt, Wilkins and Boone, *Am. J. Dis. Child.*, 1924, xxviii, 574.

¹ Lee, F. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1925, xxii, 335.

same degree as the mucosa elsewhere when pressure was applied during the injection. Finally, an increase in thickness of the muscular coats at the tubo-uterine junction was present in some animals, particularly in the rabbit.

223 (2746)

I.

Concerning the influence of polarized light on some convulsant drugs: A contribution to photo-pharmacology.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

In connection with a study of the effects of light on the actions of various drugs and poisons, which the author has been carrying on during the last few years—a field of science to which the term “photo-pharmacology” may be appropriately applied—the author made some extremely interesting observations on the influence of polarized light on the pharmacological action of certain drugs on animals, which it is the purpose briefly to describe in this communication.

The influence of light on the effects of certain cerebrally acting convulsant drugs in rats was investigated. The drugs studied in particular were camphor, santonin, and cocain. A number of experiments were also made with solutions of carbolic acid. The above drugs were injected either subcutaneously or intraperitoneally in equivalent doses in white rats, and the onset, severity and duration, etc., of the convulsions were noted under different conditions. Equivalent quantities of a drug per body weight were injected simultaneously into two or more rats. One of the animals was exposed to *polarized* light, while the other was illuminated by a *non-polarized* light of the same intensity, the temperature and other conditions being the same in the two sets of experiments. The source of light used was electric Mazda lamps of 150 or more kilowatts. Polarized light was obtained in some experiments by means of a large Nicol prism, while in

others by the well known physical expedient of allowing rays of light to fall on a pile of glass plates at the proper ("polarizing") angle. The latter method was the more convenient one for the present investigation.

It was found that after injection of camphor (in the form of camphorated oil, U. S. P.), of santonin (in the form of solutions of sodium santoninate), and of cocain hydrochloride, the convulsions came on much more quickly and were of greater violence in the rats exposed to polarized light, than in those rats which were injected with the same quantities of the same drugs and exposed to non-polarized light. In many experiments the animals under polarized light died much sooner than the controls; and indeed some of the control rats survived the effects of the poison. The following protocols will serve as illustrations:

Experiment 1, June 12, 1924.

A. Rat weighing 175 gm.

4:18 P. M. Injected intraperitoneally 1 cc. of camphorated oil and exposed to *polarized light*.

4:25 Violent epileptiform convulsions.

4:30 Died.

B. Rat weighing 165 gm.

4:16 P. M. Injected intraperitoneally with 1 cc. of camphorated oil and exposed to ordinary non-polarized light of same intensity and at the same temperature.

4:28 Slight twitching of jaw muscles.

4:42 Convulsions.

5:00 Still alive.

Experiment 2, June 12, 1924.

A. Rat weighing 210 gm.

Injected 1 cc. of camphor oil intraperitoneally. Exposed to polarized light.

Violent convulsions in 30 minutes.

Dead in one hour.

B. Rat weighing 210 gm.

Injected 1 cc. camphor oil intraperitoneally. Exposed to non-polarized light.

First convulsions noted in 45 minutes.

Rat alive after 2 hours.

Experiment 3, June 17, 1924.

A. Rat weighing 185 gm.

11:28 A. M. Injected 10 mg. cocain hydrochloride (1 per cent solution) intraperitoneally. Exposed to polarized light. Temperature 30.5° C.

11:34 Trembling and weak.

11:36 Collapse.

11:43 Violent convulsions and death.

B. Rat weighing 185 gm.

11:29 A. M. Injected 10 mg. cocain hydrochloride (1 per cent solution) intraperitoneally.

11:37 Slight excitement.

11:45 Apparently normal condition.

12 M. No change.

4:30 P. M. No change.

Next day; recovered.

Similar results were obtained with santonin, and to some extent with solutions of phenol. Most of the above experiments, some fifty in number, were performed in the spring and early summer of 1924. Protracted illness of the author necessitated a temporary discontinuance of the experimentation. It is expected to resume the investigations. The expenses of this research were defrayed in part by a grant from the Rockefeller Institute for Medical Research.

224 (2747)

II.

The influence of polarized light on the action of some ferments:
A contribution to photo-pharmacology.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

E. S. Semmens¹ reported some very interesting observations on the influence of polarized light on the action of diastase. Studying starch granules with a microscope Miss Semmens noted that, when these were illuminated by light passing through a Nicol

¹ Semmens, E. S., *Nature*, 1923, iii, 49.

prism, the conversion of starch into sugar took place more rapidly than in ordinary light. The present author experimenting on the biological effects of polarized light repeated these studies on diastase by a different method, and studied, furthermore, some other enzymes.

The effect of polarized light on the action of diastase was studied by using solutions of starch, adding definite quantities of taka-diastase solution to the same, and noting chemically, by the iodine reaction, the rapidity of diastatic enzyme action, in the dark on the one hand, and in polarized and non-polarized lights at the same temperature on the other hand. Polarized light was obtained through a large Nicol prism in some experiments, and by means of a pile of glass plates in others. The results obtained corroborated fully the observations of Miss Semmens. The conversion of starch into sugar took place more rapidly in polarized light than in non-polarized light of the same intensity.

Two other ferments were studied in this connection: rennin and catalase.

The rapidity of milk coagulation after addition of equal quantities of rennin to given amounts of milk was studied in polarized and non-polarized light. It was found that the clotting of milk took place more rapidly in polarized light than in non-polarized light of the same intensity and at the same temperature.

The effect of polarized light on the activity of blood catalase was studied also. In this case no constant difference was found in the catalase reaction of samples of blood exposed to polarized light and to non-polarized light.

225 (2748)

III.

The influence of polarized light on yeast and bacteria.

By DAVID I. MACHT and JUSTINA H. HILL.

[From the Pharmacological Laboratory and the Brady Urological Clinic, Johns Hopkins University, Baltimore, Md.]

In connection with the experiments on the biological effects of polarized light described in the preceding communications, a

study was made of the influence of such light on yeast and bacteria.

A suspension of ordinary baker's yeast, *Saccharomyces cerevisiae*, was made, and equal quantities of such a suspension were added to solutions of sucrose in fermentation tubes. Such tubes were exposed to polarized light, and also to non-polarized light of the same intensity and at the same temperature. It was found that the fermentation of sugar in polarized light proceeded much more rapidly than in non-polarized light as indicated by the amount of carbon dioxide gas evolved in the fermentation tubes.

Following the experiments on yeast a number of other experiments were begun on smaller microscopic plants, namely bacteria. Cultures of *B. coli* and *B. typhosus* were exposed to polarized light, and also to non-polarized light of the same intensity and at the same temperature. While the number of experiments with bacteria so far has not been very great, the results obtained seem to indicate that the bacterial cultures grow more profusely in polarized light. These experiments will be continued on a larger scale but it was deemed advisable to announce the results obtained so far, in view of the most interesting communication published recently by T. F. Morrison of Princeton University,¹ concerning the effect of polarized light on the growth of certain luminous bacteria. This author found that such bacteria flourished better under polarized light. If the above observations should be confirmed by further repeated experiments the results obtained would be of great hygienic interest, showing from a new point of view the importance of out-door sunlight in the treatment of certain infections, as sunlight passing through windows is always more or less polarized.

¹ Morrison, T. F., *Science*, 1925, lxi, 392.

226 (2749)

Does the optic nerve of the frog tadpole regenerate after section?

By WILLIAM H. COLE.

[*From the Biological Laboratory of Clark University, Worcester, Mass.*]

During the study of conjunctival regeneration in frog tadpoles (*Rana clamitans* and *R. catesbeiana*) it was noted that, after removal and immediate replantation of the eye, degeneration of the eye took place. The eyeball became whitish and opaque as seen through the conjunctiva, and gradually became smaller until no trace of it was visible externally. Instead of the usual convexity in the eye region, the area became depressed. It was therefore concluded that the eye was absorbed, due to the lack of regeneration of the blood vessels, muscles and nerves of the eye. Out of 37 animals on which such an operation had been performed, there was one whose eye did not degenerate. At no time did the eye become whitish or opaque, although it diminished in size about one third. For the first few weeks its position was abnormal, the eye having rotated anteriorly about 20 degrees. Gradually this displacement disappeared, and at the end of two months the eye was normal in position, although still about one third smaller. Near the end of the third month compensatory movements of the eyeball reappeared. When the body was rotated the eye always rotated in the opposite direction, as in unoperated animals. This led to the assumption that the ocular muscles must have regenerated. The fact that the eye remained normal in appearance made it seem likely that the optic nerve and blood vessels had also regenerated. During the fourth month the head was removed, fixed and sectioned for histological study. The sections were nearly transverse to the optic nerve and gave evidence that the nerve was continuous. Just posterior to the eyeball there was an irregularity in the nerve involving a slight enlargement in diameter. Since the crucial sections were not as clear as desirable, and since a longitudinal cut would have been preferable, the conclusion that the optic nerve had regenerated could be only tentative. Accordingly further operations are being made in the hope of answering the question raised.

227 (2750)

Effects of inoculating monilia isolated from psoriatic patients into human beings.

By MOYER S. FLEISHER.

[*From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine, St. Louis, Mo.*]

It has been shown by Fleisher and Wachowiak¹ that monilia or monilia-like organisms were found in 85 per cent of the stools of psoriatics, that similar or identical organisms could be cultivated or demonstrated in the skin scrapings in 35 per cent of cases, and that these organisms were present in the blood in 14 per cent of cases. However, in normal individuals such organisms were found in only 6 per cent of the stools, and in our series never on the skin or in the blood. Furthermore, the injection of an emulsion of the killed organisms led, in a certain number of intractable cases, to a startling and rapid clearing of the lesions. Efforts had also been made to reproduce the disease (skin lesions) in rabbits, guinea pigs and dogs, but without success. We believed that this evidence suggested very strongly that there exists some etiological relationship between the monilia and the occurrence of psoriatic lesions.

Recently I have studied the effects of applying the monilia isolated from cases of psoriasis to abraded surfaces of the skin of human beings.

In normal individuals, two small areas, 2 to 3 mm. square on the upper arm were denuded of the superficial layers of epidermis. One area served as control and this healed rapidly, being practically indistinguishable from the surrounding uninjured skin, after about eighteen to twenty days; at no time was desquamation or scaling noted on or about these control areas. To the other areas there was applied a drop of a mixed emulsion of live monilia. Here a scab formed which persisted for about two weeks, and usually during this period there was a varying degree of itching. In the second week scaling usually appeared about the scab. After the scab fell off there was left a dull reddened area, the redness persisting until the end of the third week

¹ Fleisher and Wachowiak, *Arch. of Dermat. and Syph.* In press.

or a little later. This area (larger than the scarified area) showed in every case a scaling or desquamation through the fourth week and in some cases even longer. After the fifth or sixth week practically all traces of the inoculation had disappeared.

Monilia were isolated from the scales from two of the eight inoculated individuals after eighteen and twenty-one days respectively; at later periods the organisms could not be demonstrated.

It appears then from these experiments that monilia may stimulate the epidermis to an activity which at least in part simulates that noted in psoriasis.

228 (2751)

Physical-chemical changes of the blood in thyroidectomized guinea pigs.

By C. M. WILHELMJ and MOYER S. FLEISHER.

[From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine, St. Louis, Mo.]

As far as we have been able to learn, no studies have been made of the surface tension of the blood in thyroidectomized animals. In the course of a series of experiments it became essential to study the surface tension of the plasma of such animals.

We removed the thyroids of a number of guinea pigs and determined the surface tension of the plasma at various periods after operation.

We used the DuNouy Tensiometer for making surface tension determinations. The animals were bled from the carotid artery through a cannula into tubes containing one per cent sodium oxalate. One part of oxalate solution to nine parts of blood was used throughout the entire series. Several experiments were made to determine the effect of different quantities of oxalate solution, and these showed that, within reasonable limits, dilution of blood with varying quantities of oxalate did not appreciably alter the surface tension.

In thyroidectomized animals we found a rise in the surface tension of the plasma, progressively increasing with the longer post-operative period. This rise reached its maximum in from twenty-two to twenty-eight days.

Plasma obtained from animals six and thirteen days after operation showed no appreciable increase; we can therefore eliminate the operative procedure as a factor in the changed surface tension. On the eighteenth day after operation the increased surface tension became evident. From the eighteenth to the twenty-eighth day there is a distinct increase in the number of animals showing high surface tension. The average surface tension of all thyroidectomized animals on or after the eighteenth day was 57.7 dynes; the average surface tension of the corresponding normal animals was 53.8 dynes. Thus the plasma from sixteen out of twenty-seven operated animals was found to be above 55.4 dynes; on the other hand 23 out of 26 normal plasmas were below this figure. Between the twenty-second and twenty-eighth days, fourteen out of seventeen operated animals had plasmas showing a surface tension considerably higher than that in an equal number of controls done simultaneously. The average difference in individual groups (controls compared with thyroidectomized animals) varied from 5.1 to 5.7 dynes.

There are probably many compensatory factors involved which tend to prevent this increase in the thyroidectomized animals, so that variation may occur in different animals. Thus some plasmas have already reached the maximum in from eighteen to nineteen days after operation, while others are occasionally found within normal limits even after twenty-four days. Our findings so far suggest that this is largely a factor of the time element in the individual animal.

In one small series, examined twenty days after operation, we used serum in place of plasma; the difference between the operated and control animals was distinctly less marked than when plasma was used.

A few experiments were undertaken to ascertain whether this difference between the plasma from normal and operated animals was in any way related to the length of time that the plasma was allowed to remain in contact with the cells. It was found that there was little or no difference between the surface tension of plasma immediately separated and the plasma allowed to remain in contact with the cells for two to five hours.

Viscosity determinations of the plasma are now being carried out, and the results so far seem to indicate an increased viscosity of the plasma of the operated animals; however, additional experiments are required before a definite statement can be made in this regard.

229 (2752)

Epinephrin anhydremia and its relation to the emergency function of the adrenals.

By H. G. BARBOUR and W. F. HAMILTON.

[*From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Louisville, Ky.*]

It has been well known, since Lamson¹ first worked on this subject, that the blood concentrates in response to small increases in its epinephrin content. This phenomenon has been studied very little in unanesthetized animals, no attention having been paid to the threshold dose of epinephrin which produces anhydremia.

We have performed 25 experiments in which epinephrin has been injected in varying amounts in a number of unanesthetized dogs. In most of these the total blood solids were determined before each injection, and at five minute intervals thereafter. To insure observation of the maximum effect, ten of the experiments were controlled by following the specific gravity (Falling Drop Method²) at approximately one minute intervals. It was found that the threshold dose for epinephrin anhydremia is approximately .0001 mg. per kilo animal, while to produce the maximum effect about .01 mg. per kilo is required. With this latter dose the greatest effect was a change of specific gravity in one case from 1.0504 to 1.0562, which occurred within 2 minutes after the injection. The normal level of blood concentration is always regained within about 10 minutes, and a definite although slight dilution often follows. It is thus seen that in unanesthetized

¹ Lamson, P. D., *J. Pharmacol. and Exper. Therap.*, 1915, vii, 169.

² Barbour, H. G., and Hamilton, W. F., *Am. J. Physiol.*, 1924, lxix, 654..

animals, this anhydremia is of rapid onset and immediately reversible. It is, therefore, unrelated to the delayed hematopoietic polycythemia described by Edmunds and Stone.³ The low dosage which suffices to evoke epinephrin anhydremia indicates that the latter is a factor which must be accounted for in all experiments bearing upon the emergency function of the adrenals.

Further evidence that blood concentration can not be overlooked in the study of emotional responses is seen in excitement anhydremia. The time, course and extent of this phenomenon closely resemble the epinephrin effect. To what extent this is referable to the adrenals, as suggested by Lamson,¹ we have attempted to determine by the expedient of cutting both splanchnic nerves in a series of five dogs. The effect upon the blood concentration of brief periods of teasing such animals before and a week or more after this operation is shown in the following table:

Excitement Changes in Total Solids or Specific Gravity of Blood Before and after Splanchnotomy.

Dog No.	Unoperated		Splanchnotomized	
	Calm	Excited	Calm	Excited
1			18.2%	18.1%
2	18.8%	22.3%	16.2%	17.3%
7	1.0503	1.0520	1.0448	1.0487
16	1.0425	1.0487	1.0398	1.0438
17	1.0497	1.0518	1.0439	1.0472

Excitement anhydremia, so far as one can judge from the above results, is of the same order of magnitude after double splanchnotomy as before. It does, therefore, not require the sympathetic control of liver, spleen or adrenals. Any of these may of course, in normal animals, participate in the emergency concentration of the blood.

³ Edmunds, C. W., and Stone, R. P., *Arch. internat. de Pharm. et de Therap.*, 1924, xxviii, 391.

230 (2753)

The effect of high protein diets on the kidneys of rats.

By HENRY JACKSON, JR. (Introduced by Francis W. Peabody).

[*From the Thorndike Memorial Laboratory, Boston City Hospital, and the Department of Medicine, Harvard Medical School.*]

As a part of a series of experiments designed to study the etiology of chronic nephritis, a number of rats were placed on diets made up of pure food materials, pure salts and vitamins. Diet A contained 20 per cent casein by weight, and was used as the standard or base diet. Diet B contained 76 per cent casein. Diet C contained 76 per cent casein plus the calculated amount of sodium bicarbonate necessary to neutralize the phosphorus and the sulphur derived from the casein. Diet D contained by weight, 20 per cent casein and 56 per cent purified egg albumen.

The rats, all of which came from the same stock, were, at the beginning of the experiment, from 50 to 300 grams in weight. They were kept on the various diets for 8 to 14 months.

Those on standard diet consumed 0.97 grams protein per 100 grams rat body weight per day. Those on Diets B and C ate 3.65 grams protein per 100 grams rat per day; those on high egg albumen Diet D, 3.60 grams. Figured in calories, the standard series ate 21.4 calories per 100 grams body weight per day; the high protein series, 21.2 calories; and the high egg albumen, 20.8 calories.

All the rats grew normally. The growth curves on the various diets were similar and agreed with the standard curves of other observers. Young rats grew rapidly and maintained their growth well on all diets.

At various intervals the urine was tested for albumen, the sediment was examined, and quantitative nitrogen determinations were made. Traces of albumen were found in the urine of all our rats, in this and in other series as well. In but one instance did the albumen increase during the progress of the experiment, and that was in a rat which died of a chronic lung disease. Casts were not found in any case except in the same individual. No qualitative or quantitative abnormalities which might be attributed to the high protein were found in the nitrogen metabolism. The non-protein nitrogen of the blood of the standard diet rats

at the time of death varied from 30 to 40 mg. per 100 cc. whole blood. The non-protein nitrogen of the rats on high protein and high albumen diets varied from 40 to 62 mg. per 100 cc. No clinical signs of nephritis were found.

The rats were autopsied, and the organs were studied grossly and microscopically. With the exception of a pseudo-tuberculous lesion in the lungs of some rats, no pathology was found.

The kidneys of the rats on high protein diets were considerably hypertrophied. Microscopically there were no lesions in the glomeruli, tubules or interstitial tissue. Occasional casts were found in the kidneys of rats on alkali diet.

We have been unable to produce nephritis of any sort in rats by high protein diets over a long period of time.

231 (2754)

Extractives of liver possessing blood pressure reducing properties. Report of clinical tests.

By W. J. MACDONALD. (Introduced by John R. Williams).

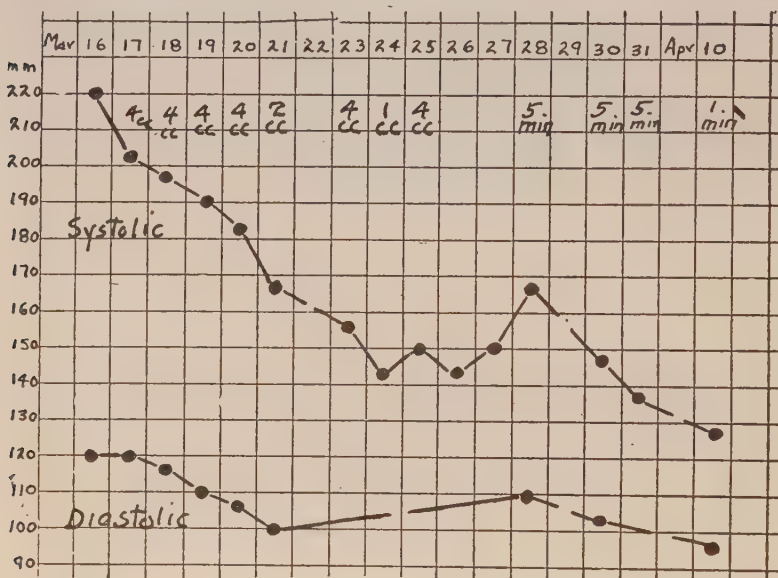
[*From the Department of Biochemistry, London, Ontario, Medical College and MacGregor-Mowbray Clinic, Hamilton, Ontario.*]

In January, 1923, I became interested in the effect of certain extracts of the liver on the metabolism of uric acid in dogs. While this investigation did not yield information of significance, it was observed that the intravenous injection of these products possessed depressor principles which caused a sharp and fairly persistent fall in both systolic and diastolic blood pressures.

It is quite generally known that various fragments of protein cleavage, notably histamine and the guanidine salts, possess depressor and pressor properties; and that various proteins, when injected into the circulation, induce shock and accompanying fall in blood pressure.

The theory underlying the experiments here reported is that the liver structure may contain an active principle which will regulate blood pressure.

Preliminary tests indicated that the extracts employed were non-toxic to dogs. It was therefore decided to use the material on individuals known to be suffering from persistent and long standing arterial hypertension. The first tests were made on cases who presented no gross physiological or metabolic defects other than hypertension. In later tests the patients were more critically studied with reference to associated disease phenomena.



Curves showing systolic and diastolic pressures in a case of essential hypertension. Patient, female, age 45 years. Known duration of hypertension, 2 years. Previous systolic range 200-240 mm. Previous diastolic range 120-130 mm. In 1923 patient had a slight hemiplegia from which she made a good recovery. From March 21 to March 28 diastolic pressure difficult to obtain.

Total number of cases observed	33
Range of age of patients	45-67 yrs.
Average age	61 yrs.
Average known duration of hypertension in years	6
Average range of systolic pressure before injection.....	204 mm.
Average range of systolic pressure after injection.....	142 mm.
Average fall in systolic pressure	62 mm.
Average range of diastolic pressure before injection.....	114 mm.
Average range of diastolic pressure after injection.....	86 mm.
Average fall in diastolic pressure	28 mm.
Cases where no reactions occurred	25
Number of cases experiencing reaction	8

It is the purpose of this paper to report blood pressure observations on a series of cases of arterial hypertension in which there has been a fall in both systolic and diastolic pressures, following the administration of certain extracts prepared from the liver. In all, 33 cases have been studied. The procedure of administration has been as follows: A normal salt solution of the extracts of varying dosage was injected intravenously. In 25 cases the patients experienced no disagreeable symptoms, most of them reporting apparent relief. In 8 cases there followed reactions of varying degrees, some of which closely resembled protein shock, namely, chill, rise in temperature lasting several hours, nervousness, loss of appetite and weakness, together with a sharp fall in both systolic and diastolic pressures. Of these reacting cases five were studied in the Highland Hospital, Rochester, with Dr. J. R. Williams. The shock phenomena were observed only in cases treated from one batch of extract. In the cases free from symptoms, one injection served to depress the blood pressure from 1 to 3 or more days, according to size of dose. In all of the favorable cases, the return to hypertension was gradual and rarely to the former level.

The following physicians made independent observations on selected cases, and their results are included in this report: Drs. Mullin, Tice, Park, McGhie, Pain and Farmer of Hamilton; Dr. Bertram of Dundas; Dr. Williams of Rochester.

The chemical character of the extract has not yet been identified. Preliminary chemical study suggests that it is not histamine. It is possible that the effects observed are due to protein shock, but this latter phenomenon is usually briefer in action (a matter of hours) and accompanied by quite marked physiological reactions; whereas in most of the cases observed in this study, the depressor effect was sustained for one or more days, and without noticeable reactions. Chemical and physiological studies are now in progress in the further investigation of the problem. These clinical tests would indicate that the liver possesses a principle which may be of use in the control of certain cases of arterial hypertension.

232 (2755)

Gasometric determination of urea with urease.

By DONALD D. VAN SLYKE.

[From the Hospital of the Rockefeller Institute for Medical Research, New York City.]

Urea is changed by urease into ammonium carbonate. The ammonia is commonly determined as a measure of the urea. However, as shown by Partos¹ and by Mirkin,² one can also estimate the urea from the CO₂ formed. The manometric blood gas apparatus of Van Slyke and Neill³ is particularly adapted to this determination, because of the wide range over which it yields accurate results.

Blood. The entire estimation can be carried out in the apparatus, as the blood proteins protect the urease from inactivation by the mercury. For a micro-determination, 0.2 cc. of blood and 1 cc. of 0.02 N lactic acid are placed in the apparatus, which is evacuated and shaken 1 minute to remove preformed CO₂. Five-tenths cc. of 10 per cent urease solution⁴ (Squibb) and 0.1 cc. (3 drops) of CO₂-free 0.3 M Na₂ HPO₄ solution are then added. Mercury in the chamber is lowered and raised again, to bring the urease into contact with the blood solution wetting the walls. The mixture is allowed to stand 5 minutes or more for decomposition of the urea. Three drops of N/1 lactic acid are then added, plus enough water to bring the total solution to 2 cc. The CO₂ is then extracted by 1½ minute shaking, and determined, as described by Van Slyke and Neill for micro CO₂ determination in 0.2 cc. of blood. A concentration of 1 millimol of CO₂ per liter of blood is equivalent to 1 millimol or 60 mg. of urea, per liter.

For 1 cc. portions of blood, 1 cc. of 0.1 N lactic acid is used to remove preformed CO₂. The amount 0.3 M Na₂ HPO₄ is increased five-fold to 0.5 cc.; the enzyme to 0.5 cc. of 20 per cent urease; and after digestion 0.5 cc. of 1 N lactic acid is added.

¹ Partos, S., *Biochem. Ztschr.*, 1921, ciii, 292.

² Mirkin, A., *J. Lab. Clin. Med.*, 1922, viii, 50.

³ Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

⁴ Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, xix, 211.

The CO_2 determination is performed as described by Van Slyke and Neill for 1 cc. blood samples.

Urine. In each of two 20 cc. cylinders, A and B, place 1 cc. of urine (or 2 cc. if the urine is dilute). To each cylinder add 5 cc. of a buffer solution containing per liter 4 grams of KH_2PO_4 and 2 grams of Na_2HPO_4 . Dilute B to 20 cc. and A to 19 cc. and cover both with 3 or 4 cc. of paraffin oil. To A add 1 cc. of 10 per cent urease. Stir each solution gently by moving a footed rod in it up and down a few times. Let stand 30 minutes for enzyme to act. Determine CO_2 in 2 cc. samples from each solution, adding 1.5 cc. of 0.1 N lactic acid to decompose the carbonate, so that the total volume of solution in the blood gas apparatus is 3.5 cc. The CO_2 is determined as described by Van Slyke and Neill for 1 cc. of blood, except that absorption of CO_2 with alkali is unnecessary. The total gas pressure only is measured, the reading from A giving p_1 that from B giving p_2 , for calculation as described by Van Slyke and Neill.

The determination can also be performed with the original volumetric apparatus of Van Slyke, although the results cannot be read with as great accuracy.

The entire blood determination requires about 15 minutes, the analysis of solutions A and B in the urine determinations 5 minutes each. The gasometric method has the advantages that aeration and distillation are avoided, and that standard solutions for titration or colorimetric comparison are unnecessary.

233 (2756)

A method for obtaining distribution of a therapeutic agent throughout the intestinal tract.

By JAMES W. JOBLING.

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Treatment of infections of the lower ilium and colon by the oral administration of drugs has been unsatisfactory, owing to the difficulty of getting the drug to these regions unchanged. Tablets coated with various substances have been used, but with poor results.

In the course of work which we have been doing during the past two years, a method has been developed which permits us to obtain a fairly uniform distribution of a chemical substance throughout the intestinal tract.

Gentian Violet has been used in most of our experiments. Gelatin capsules were filled with a mixture composed of the dye and sodium chloride or a buffer, and then coated with 3 per cent collodion. In our preliminary work we found that Gentian Violet will not dialyze through such a membrane, so a minute hole was made in one end of the capsule with a No. 12 cambric needle. The sodium chloride and buffer mixtures were used to raise the osmotic pressure and to neutralize any acids or alkalies which might be present. Following the entrance of fluid into the capsule the gelatin dissolves, leaving the thin collodion layer which collapses from the pressure of the intestinal contents. This aids in causing the expulsion of the dye. Capsules recovered from the feces are intact and usually empty.

When capsules prepared in this manner are administered to animals or human beings, the feces are uniformly colored with the Gentian Violet. We have had two autopsies on individuals who had been receiving the capsules, and in both instances the contents of the gastro-intestinal tract from the mouth to the anus were stained a deep violet. The mucosa throughout was also stained.

234 (2757)

Experimental production of intra-ocular hypertension.

By THEODORE KOPPÁNYI and THOMAS DYER ALLEN. (Introduced by A. J. Carlson).

[*From the Hull Physiological Laboratory of the University of Chicago, Chicago, Ill.*]

It is commonly believed that the intra-ocular tension is sustained by the blood pressure in the intra-ocular blood vessels, especially by those in the iris; and that it may be lowered either by fall of the general blood pressure, or by constriction of the vessels locally. Results achieved following administration of drugs have not been uniform; for instance, after cocaine, the intra-

ocular tension may remain unchanged or may be raised or lowered.

We measured the intra-ocular tension with the Souter-tonometer and later with the mercury manometer, by insertion of a needle subconjunctivally into the anterior chamber. All drugs were injected into the common carotid artery. The experiments were made on dogs under ether anesthesia.

After injection into the carotid artery of 5 minims or more of chloroform, we several times noticed forced position of the eyes, (a) enophthalmos, (b) exophthalmos, (c) deviation of the globe. At the same time there was an hemolysis, edema of the retina, retinal and iridic hemorrhages, and increase in the intra-ocular tension to plus 3.

Following this administration of chloroform the general blood pressure falls, and a local vasoconstriction occurs in the retina, the two factors, which it is believed, ordinarily are followed by a lowering of the intra-ocular pressure. We found, however, without exception, that the intra-ocular tension rose from a normal of 15 to 23 mm. Hg. to 70 or more mm. of Hg. in the eye on the side of the injection, returning to 40 mm. of Hg. in 20 minutes, and gradually decreasing to normal. There was no change in the depth of the anterior chamber.

Ether.—2 cc. was followed similarly by immediate blanching of the retinal vessels and increase in tension. There were these differences: (a) increase in tension is not quite as rapid as with chloroform (tension rose to 70+ mm., however); (b) there are not as marked changes in the eye, although hemorrhages were occasionally seen.

Concentrated sugar solutions produced an increase in tension to 50 mm. Hg.

Adrenalin produced a slight increase in the intra-ocular tension.

Alcohol 40 per cent—4 cc. produced a lowering of the tension from 15 to 23 down to 10 mm. or less.

Quinine-urea-hydrochloride, calcium chloride, saturated saline solution, tap water, hot and cold, did not produce any appreciable changes in the intra-ocular pressure.

235 (2758)

Nervous and pharmacodynamic control of the retinal blood flow.

By THOMAS DYER ALLEN and THEODORE KOPPANYI. (Introduced by A. J. Carlson).

[*From the Hull Physiological Laboratory of the University of Chicago, Chicago, Ill.*]

The literature on the vasomotor control of the retinal blood vessels, especially the arterioles, is conflicting. Several authors report that the nervous control of the size of the blood vessels of the retina is in the cervical sympathetic, as is the case in the vessels of many of the other organs of the head. In the rabbit there is agreement, apparently, that the cervical sympathetic controls the size of the retinal vessels. In the higher mammals, however, especially in the carnivora, while some authors report similar control, other investigators obtained negative results. From a practical standpoint as well as from a theoretical one this is an important question; for in man we not infrequently see embolism of the central artery of the retina; and we are constantly seeking further light on the management of these cases.

Our work so far has been done on dogs. We found that stimulation with the faradic current of the central end of the vagus-sympathetic trunk, as a rule, produced narrowing of the retinal arteries and veins. The examination was always made by means of the electric ophthalmoscope. We made attempts to isolate the sympathetic nerve by following the nerves to their ganglia. The stimulation of the isolated sympathetic produced the same results as stimulation of the vagus sympathetic trunk. Section of the cervical sympathetic is followed by slight dilatation of the retinal arteries. In some animals, however, we were unable to see any effects following stimulation or cutting of the cervical sympathetic.

Stimulation of the internal carotid plexus produced marked reduction in the size of the retinal arteries of the same side. Stimulation of the central end of the sciatic nerve produced constriction of the retinal vessels, provided the cervical sympathetic nerves were intact.

During the course of our investigation we were able to confirm

an older observation that compression of the trachea (asphyxia) produces dilatation of the retinal veins.

Inhalation of amyl nitrite from a pearl broken into the mouth produced dilatation of the retinal arteries; in one case intermittent dilatation and constriction of the arteries was noted.

Chloroform anesthesia produced no noticeable change in the blood stream in the retina. Nor could we see any changes following intravenous administration of strychnine. Adrenalin produced a noticeable narrowing of the blood vessels in some cases. We failed to obtain a visible dilatation of the arteries as noted by an earlier observer. Adrenalin administration was followed by the same result as stimulation of the cervical sympathetic.

Late in several experiments, when the blood pressure was low, a moderate pressure on the eye, as in closing the eyelids and opening them, produced blanching of the retinal vessels, with rapid return of flow.

Different drugs were injected into the common carotid, usually on the same side as the eye investigated. The solutions were frequently stained with methylene blue, and the stain observed in the fundus.

Two to three minims of chloroform injected into the carotid caused slight dilation of the retinal vessels. *Strong contractions* of the retinal vessels were produced by 5 min. chloroform, by 2 cc. ether, 1 cc. quinine-urea-hydrochloride, and 3 cc. 40 per cent dextrose.

Four cc. 40 per cent alcohol produced at first a slight dilatation of the retinal arteries; this was followed almost at once by very marked constriction lasting about 10 to 15 minutes.

Nitroglycerine, 1/50 gr. produced a slight dilatation of the retinal arteries, and constriction of the pupil.

A number of other fluids, like warm (40-60° C.) or cold (15-25° C.) water, or calcium chloride solution, did not seem to have any effect on the size of the vessels.

WESTERN NEW YORK BRANCH

*Syracuse University Medical School, Syracuse, N. Y.**April 25, 1925.*

236 (2759)

Inactivation and reactivation of insulin.

By R. S. ALLEN and J. R. MURLIN.

[From the Department of Vital Economics, University of Rochester, Rochester, N. Y.]

That insulin may be inactivated by treatment with reducing and oxidizing agents has been demonstrated by Shonle and Waldo.¹ They found that, when insulin was treated with a dilute solution of hydrogen peroxide or potassium permanganate, its glucopyretic property was entirely destroyed. They likewise observed that the activity was destroyed by action of such reducing agents as sodium bisulphide, sulphur dioxide, hydrogen, and stannous chloride. They were never able to recover the activity after it had once been lost by either oxidation or reduction as indicated. Dodds and Dickens² observed that the activity of insulin prepared by the picrate method was reduced and finally lost if subjected to formalin in various concentrations. Complete inactivation resulted from exposure to 10 per cent formalin at 37° for one hour. Destruction was much less rapid at a lower temperature. Dodds and Dickens believe that the inactivation was due to combination of formaldehyde with free NH₂ groups of the insulin protein. However, formaldehyde is a fairly good reducing agent and we suggest that the destruction of the glucopyretic property of insulin may equally well be accounted for by a reducing effect upon the insulin molecule. We have observed that fairly pure insulin products, as prepared by our acid aqueous extraction with heat, precipitation by sodium chloride, and purification by repeated reprecipitation with amyl alcohol³ is extremely sensitive to oxidation or reduction. This was first noticed quite

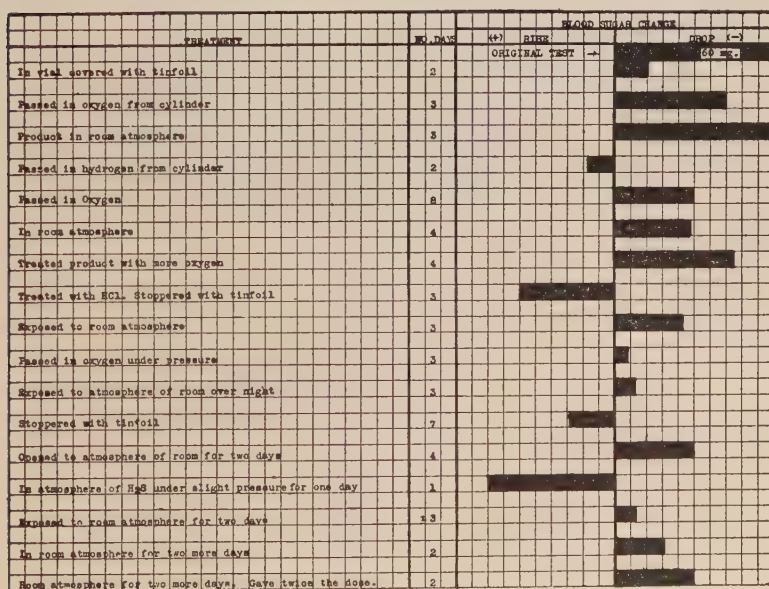
¹ Shonle, H. A., and Waldo, H., *J. Biol. Chem.*, 1924, lviii, 736.

² Dodds, E. C., and Dickens, F., *The Brit. J. Exper. Path.*, 1924, v, 117-118.

³ Allen, R. S., Piper, H. A., Kimball, C. P., and Murlin, John R., *Proc. Soc. Exp. Biol. and Med.*, 1923, xx, 519-521.

by accident when a dried amyl alcohol precipitate was closed up in a vial, the stopper of which was covered with tinfoil. The precipitate had been dissolved in weak hydrochloric acid at an earlier stage, and it was noticed that the tinfoil became very black, due to action of the volatile acid. When the product was again tested, it was found to be almost devoid of potency. Removal of the stopper and exposure to air restored the potency. Proof that the inactivation was caused by free hydrogen was furnished by exposure to the commercial gas. A second and third time it was inactivated with hydrogen from tinfoil, and again restored by exposure to air or exposure to oxygen under pressure. It was also inactivated by exposure to hydrogen sulphide, and then restored by exposure to air. What seems even more significant is that the reduced insulin on several occasions raised the blood sugar of rabbits very materially. Prolonged exposure to oxygen under pressure on one occasion destroyed a large part of the potency, but did not change the glucopyretic action to a glucagetic action. The chart gives the history of a single preparation.

INACTIVATION AND REACTIVATION OF INSULIN.



All blood sugar tests were made in duplicate on rabbits fasted 18 hours, second blood was taken in two hours. In the chart each block of the abscissal distance represents 6 milligrams rise or fall.

237 (2760)

The influence of sunlight on the mineral nutrition of swine.

By L. A. MAYNARD, S. A. GOLDBERG and R. C. MILLER.

[From the Department of Animal Husbandry and the Department of Comparative Pathology, Cornell University, Ithaca, N. Y.]

In a previous publication¹ the writers have reported a study of a condition of stiffness in swine, in which inadequate mineral nutrition was found to be a cause of the trouble. In this study the experimental animals had been so housed that they were never exposed to direct sunlight. The present paper describes a repetition of a portion of the previous experiment to study how the results might be modified were the pigs exposed to sunlight.

The ration used consisted of 200 lbs. yellow corn meal, 100 lbs. wheat middlings, and 75 lbs. oil meal—a ration very low in calcium.

In the spring of 1924, 4 pure bred Duroc pigs were placed on this ration in a pen on the north side of the colony house as in previous studies. A second group of 4 was placed on the same ration in a pen on the south side of the house opening on a cement run-way outside, 7 x 12 ft., thus giving the pigs access to direct sunlight. The experimental animals were so selected and distributed between the two pens that the comparative chemical and pathological studies could be made upon litter mates. Over an experimental period of four months, all of the pigs in the no-sunlight group developed the characteristic stiffness, while none of the pigs in the other group showed any signs of the trouble.

At the end of the four-months period the pigs were killed for routine pathological examination of the bones. On chemical analysis the femurs of the sunlight group were found markedly higher in ash content than those of their litter mates fed the same ration without sunlight.

The comparison between the sunlight and no-sunlight groups was repeated the following winter. Again, all of the pigs in the no-sunlight group became stiff, and one pig in the sunlight group became slightly stiff also. For three out of four of the pairs of litter mates compared, the ash content of the femurs of the pigs

¹ Cornell Agr. Expt. Sta. Memoir 86, 1925.

exposed to sunlight was higher than was the case with the other group, by approximately the same amount as in the previous trial. For the other pair of litter mates no difference was noted.

The results of the two trials are in general agreement in indicating that sunlight increased the assimilation of calcium in the calcium-deficient ration and prevented the development of the stiffness over the four-months experimental period.

On gross examination, the femurs of the pigs in the no-sunlight group in both trials showed lesions similar to those previously reported by the writers.¹ The cortical bone was soft and porous. The marrow in the shaft was reddened throughout or near the epiphyseal cartilage. The latter was thickened and very irregular. There were hemorrhages under the articular cartilage of the head of the femur. In one pig there was a subperiosteal hemorrhagic zone over the entire extent of the cortical bone, apparently containing spongy, osseous or osteoid tissue. The bone of the femurs of the sunlight group was much denser and was more completely calcified. There were only scattered reddened areas in the marrow of the shaft. The epiphyseal cartilage was irregular but less so than for the no-sunlight group. Thus the results of the pathological examination of the femurs were in agreement with the chemical analyses in showing that the sunlight has a marked influence on bone development.

238 (2761)

A method for the quantitative study of intestinal absorption.

By CARL F. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

In previous methods for the study of absorption, isolated intestinal loops of dogs, cats and rabbits were used, and generally one of two principles was followed: Either an acute experiment was made, which involved narcosis of the animal and a laparotomy; or a Vella fistula was established in a preceding operation. One disadvantage of these methods is that experiments made on

different animals are not strictly comparable because of the difficulty to isolate in each animal intestinal loops of exactly the same absorbing surface. Furthermore, by working only on a part of the intestine, the mechanism of absorption of the whole intestinal tract as a physiological unity cannot be studied, nor can the total absorbing capacity of the whole intestine be estimated. Finally the element of body weight cannot be taken into account. Yet it seems of importance for many problems to be able to measure and express absorption in terms of unit of body weight and hour of time, or to establish a relationship between the amount of substance that has passed into the blood stream in a given time and the body weight.

The method that is proposed below allows quantitative estimation of the absorbing capacity of the whole intestinal tract under entirely physiological conditions. So far only the absorption of different sugars has been studied. Since only small laboratory animals can be obtained in sufficient number and of the desired uniformity of stock, age and nutritional condition, the method was worked out on rats. The principle is briefly as follows: A known amount of the substance under investigation is fed by stomach tube. After a given time the animals are killed and the amount of substance remaining in the intestine is determined quantitatively. The difference between the amount fed and the amount recovered from the whole intestinal tract is then the amount of substance absorbed. Rats between two and three months of age, weighing from 120 to 180 grams, were found most suitable. They were starved for 48 hours previous to the experiments, which diminished the amount of reducing substances remaining in the intestine to a negligible minimum. Generally 1.25 to 2.5 cc. of a 50 per cent sugar solution, warmed up to 40° C., were fed. Urethral catheters Nos. 4 or 5 served as stomach tube. The rats were killed in hourly intervals after the sugar feeding, a larger group of rats serving for each 1 hour period. After placing ligatures around the oesophagus and the rectum, the stomach, small intestine and the whole large intestine were carefully detached from the mesentery, placed in a beaker and cut open. The intestine was washed out with successive portions of hot distilled water. The washings were made up to a definite volume, the interfering substances precipitated with colloidal iron, and the sugar determined in an aliquot part of the final

filtrate. Numerous control experiments were made to test the accuracy of the different steps involved in this method. If rats were killed immediately after the sugar feeding, 99.4 to 99.8 per cent of sugar was recovered.

In order to use animals of different weight, one would have to show that the absorbing surface of the intestine is proportional to the body weight, or in other words that the quotient *Intestinal surface/Body weight* or *Amount absorbed/Body weight* is a constant. Our experiments revealed that, within the range of body weights investigated, both sexes showed a proportionality between the amount absorbed (and hence between intestinal surface), and body weight. The amount of substance absorbed per 100 gram body weight in one hour has been called the absorption coefficient. The following example illustrates the constancy of the results that can be obtained with this method: 2.5 cc. of a 50 per cent glucose solution were fed to 8 rats weighing 117.7 to 173.7 grams. They were killed after one hour. The average absorption coefficient was 0.196 grams \pm 0.014 grams, or a maximum deviation from the mean of 7.1 per cent. By allowing the absorption to proceed for 2.3 hours and more, definite absorption curves can be constructed for each substance.

239 (2762)

The rate of absorption of hexoses and pentoses.

By CARL F. CORI.

[*From the State Institute for the Study of Malignant Disease, Buffalo, N. Y.*]

With the method described in the preceding abstract, the rate of absorption of the sugars referred to in Table I has been investigated. Over 100 rats have been used for obtaining the data recorded in this table. The absorption coefficients represent an average of 1, 2 and 3 hour periods in the case of galactose and glucose, and of 1, 2, 3, 4 and 5 hour periods in the case of the other sugars. It will be noted that a relatively slight change in

the sugar molecule affects the rate of absorption very markedly. The ketosugar fructose is absorbed at a rate of less than one half of that of glucose. The isomeric change from glucose to mannose reduces the rate of absorption to one-fifth. Pentoses are absorbed at still slower rates. All this indicates that the intestinal membrane is possessed of a high degree of selective permeability for sugars.

TABLE I.

Comparison of the rate of absorption of 50 per cent solutions of hexoses and pentoses.

Type of sugar.	Average Absorption coefficient.	Ratio (Glucose = 100)
d-Galactose	0.196	110
d-Glucose	0.178	100
d-Fructose	0.077	43
d-Mannose	0.034	19
l-Xylose	0.028	15
l-Arabinose	0.016	9

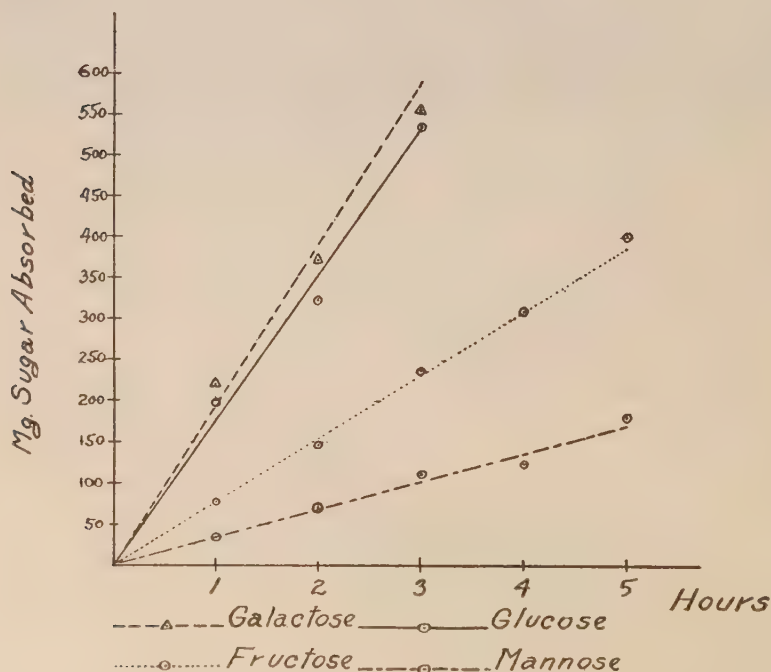


FIGURE 1.

Graphic representation of the rate of absorption of hexoses.

When the amount of sugar that is absorbed is plotted against time, a straight line is obtained, as is illustrated in Figure 1. The maximum deviation from the mean was ± 10 per cent, which is as close an agreement as can be expected from a biological method such as the one used for this work. The straight lines in Figure 1 extend to a point where about 50 to 70 per cent of the sugar originally introduced has been absorbed. From this it follows that the rate of absorption remains constant in spite of the diminution of the amount and also the concentration of sugar present in the intestine; or that the rate of absorption is independent of both factors.

240 (2763)

Quantitative studies on the pars tuberalis of the hypophysis cerebri.

By WAYNE J. ATWELL.

[From the Department of Anatomy, University of Buffalo, School of Medicine, Buffalo, N. Y.]

The *pars tuberalis* has been shown to be a distinct portion of the epithelial hypophysis both embryologically and histologically.^{1, 2} Whether it serves a distinct function, if any, has not been determined.³

The method employed has been that utilized by Hammar, Rasmussen⁴ and others. The three parts of the epithelial hypophysis were carefully outlined on paper of uniform thickness using serial sections and the projection microscope. The several parts were then cut out with scissors, and weighed. The volume of each part was determined in per cent of the total volume of the entire epithelial hypophysis. Then the value of the *pars tuberalis* in per cent of the *pars intermedia* was calculated.

In seventeen amphibia examined, there is great variation in the relative size of the *pars tuberalis* and the *pars intermedia*. In the

¹ F. Tilney, *Internat. Monatschr. f. Anat. u. Physiol.*, 1913, xxx.

² W. J. Atwell, *Am. J. Anat.*, 1918, xxiv, 271.

³ W. J. Atwell and C. J. Marinus, *Am. J. Physiol.*, 1918, xlvii, 76.

⁴ A. T. Rasmussen, *Endocrinology*, 1924, viii, 509.

tailed amphibia the *pars tuberalis* is relatively much larger than it is in the Anura. In *Plethodon gutinosus* and *P. cinereus* it is from one and a half to nearly five times as large as the *pars intermedia*.

In *Rana pipiens*, on the other hand, the *pars tuberalis* is much the smaller. In four frogs examined, the volume of the *pars tuberalis* was only $4\frac{1}{4}$ per cent of the volume of the *pars intermedia*.

The cat was selected as the mammalian type for this study. In this species the *pars tuberalis* may be distinguished with ease. The relative values for the three lobes is shown in the appended table.

Two important deductions may be drawn from our data: (1) In a number of vertebrate forms the volume of the *pars tuberalis* is equal or nearly equal to that of the *pars intermedia*; (2) As far as volume is concerned the *pars tuberalis* may be capable of producing an important secretion in forms as high as the mammals.

Relative Volume of Lobes of Epithelial Hypophysis.

Mammalia (<i>Felis domes.</i>)	Sex	Weight grams	Volume per cent of total epithelial hypophysis			<i>Pars tuberalis</i> in per cent of <i>pars intermedia</i>
			<i>Pars anterior</i> <i>proprior.</i>	<i>Pars intermedia</i>	<i>Pars tuberalis</i>	
Cat 10	Female	1181	79.17	11.67	9.18	78.66
Cat 20	Female	2000	74.83	16.58	8.41	50.72
Cat 16	Male	1880	78.92	12.24	8.85	73.30
Cat 18	Male	2400	71.03	23.24	5.72	24.61

241 (2764)

Experimental hypoglycemia and hyperglycemia in the chick embryo.

By ERNEST B. HANAN.

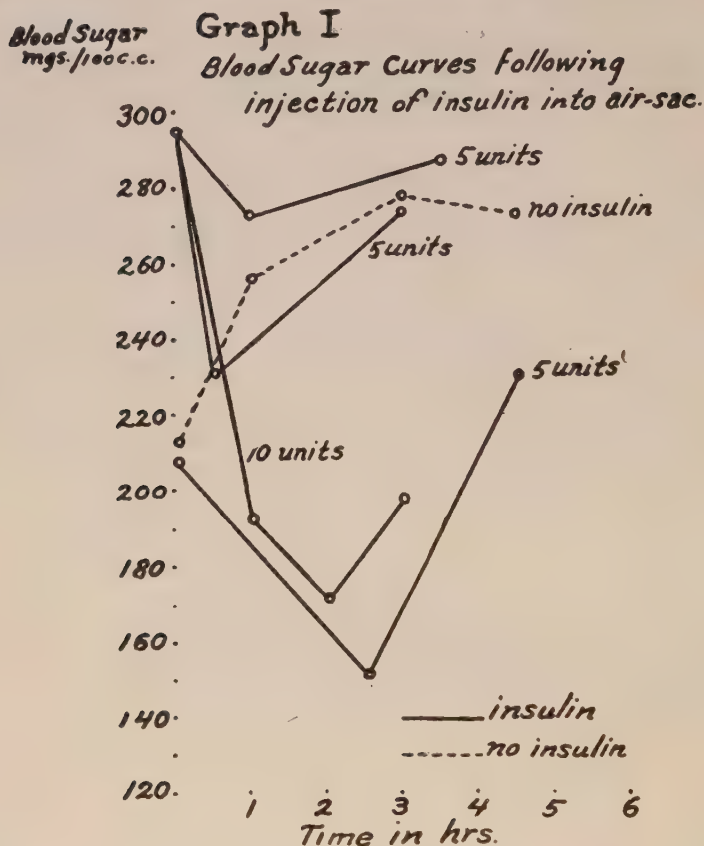
[*From the Department of Anatomy, Medical School, University of Buffalo, Buffalo, N. Y.*]

It has been found possible to produce hypoglycemia and hyperglycemia in the chick embryo. These experiments were carried out of 14 to 16 day embryos of the single combed white Leghorn variety. Immediately after the normal blood samples were taken, the injection was made into the air-sac from which absorption takes place into the blood stream of the respiratory capillary vascular network of the chorio-allantois.

The normal blood sugar of the chick embryo at this stage of incubation varies between 209 and 296 mg. per 100 cc. It appears that the glucose reaches its normally high level in birds at an early stage of development. The graph shows the effect of insulin upon the blood sugar. Riddle¹ has shown that adult birds survive thirty times the proportional amount of insulin required to kill a one kilogram rabbit. It is interesting to note that this resistance probably is established very early in the embryo. An important factor in this resistance is suggested by the hyperglycemia which is known to follow hemorrhage in the adult mammalian. That this occurs also in the chick embryo is shown by the dotted curve in the graph, obtained from an experiment in which no insulin was given. In an adult animal with a large volume of blood, the increase of sugar following blood sampling would be negligible, but in the case of the embryo with its comparatively small volume of blood, the taking of one-tenth cc. samples is a severe strain upon the embryonic physiological processes. The result is a marked hyperglycemia as shown, which would neutralize to some extent the hypoglycemic effects of insulin.

Very marked hyperglycemia may be produced by injecting a glucose solution into the air-sac as shown in Table 1. In the first two experiments of the series, the rapid clotting of the blood

¹ Riddle, Oscar, *PROC. SOC. EXP. BIOL. AND MED.*, 1923, xx, 244.



failed to occur after the taking of the second samples. In the last experiment the glucose injected was decreased to 100 mg. in one-half cc. of distilled water. At the end of the first hour gross inspection showed that it had been completely absorbed from the air-sac. The blood sugar had increased from 221 to 859 mg. per 100 cc. By the fourth hour the sugar level had fallen to practically the normal, 226 mg. per 100 cc.

The blood sugar curves in the graph were determined by the usual Hagedorn Jensen volume method² while the hyperglycemia data in Table I was obtained by a modified technique as follows: The Hagedorn Jensen procedure is standardized in milligrams of glucose per one cc. of the potassium ferrocyanide solution. In preparation for the blood sample, several one inch length are cut

² Hagedorn and Jensen, *Biochem. Ztschr.*, 1923, cxxv, 46.

from a five millimeter glass tube and are thoroughly cleansed and dried. Using fine forceps, a piece of sterile absorbent cotton just large enough to fit snugly is folded and pushed into one end of a tube so that none of the cotton fibers extend beyond either of the tube ends. The folding of the cotton as it is inserted at one end of the glass tube presents towards the other end the smooth rounded end of the cotton plug. These cotton tubes are placed in weighing tubes and set aside in a desicator for safe keeping.

To secure a sample of blood, the weight of a weighing tube with its cotton tube is carefully determined. The cotton tube is removed, and the rounded end of the cotton pushed out about one millimeter. The egg is candled and the allantoic vein at its bifurcation just below the air-sac is marked on the shell. About a square centimeter of shell is removed from over this area, and the vein exposed by turning back the shell membranes. The embryonic membranes are dried with sterile absorbent cotton, and the vein is punctured by means of a very sharp dissecting needle, using care not to puncture into the allantoic space or to produce an extensive wound. The cotton as previously prepared is brought into contact with the blood as it flows from the wound. All of the constituents of the blood are rapidly absorbed and the sample secured within a few seconds. The cotton is immediately pulled back into its glass protector and returned to its weighing tube. Several samples may be taken. The egg is sealed by Clark's method³ and returned to the incubator. The weighing tube is weighed as before, and the weight of the blood sample determined by subtraction.

Following the usual Hagedorn procedure for precipitation of proteins, the cotton blood sample is pushed into the test tube by means of a glass stirring-rod and its glass protector allowed to follow. The contents of the test tube are thoroughly stirred, and then subjected to the usual water bath for five minutes. The rest of the steps are the usual procedure.

The blood sugar in mg. per 100 cc. of blood may be calculated by the following formula:

$$\frac{1120}{\text{Blood sample in mg.}} \times \text{glucose in mg.} \times 100 = \text{mg. glucose per 100 cc. of blood.}$$

1.120 grams = average weight of 1 cc. of blood from newly hatched chick.

³ Clark, Eliot R., *Science*, 1920, li, 1319.

But the specific gravity of the chick's blood is not known for the incubation period, so that glucose should be expressed in milligrams per so many grams of blood pending such investigation.

This technique avoids the use of anticoagulants as the blood does not clot in the cotton, and it also makes unnecessary the use of oil to prevent evaporation. These advantages permit the drawing of the smallest possible samples of blood.

By means of such technique it is proposed to determine the normal blood sugar curve for the developing chick.

TABLE 1.

Hyperglycemia in chick embryo resulting from injection of glucose into air-sac.
Blood sugar in mg. per 1.120 grams of blood $\times 100$.

Age in days	Amount of glucose injected	Volume of fluid injected	Time in hours	Blood sugar	Remarks
15	400 mg.	2 cc.	0	202	Normal. Absorption incomplete. Death from loss of blood.
			1	441	
			2		
16	200 mg.	1 cc.	0	202	Normal. Absorption incomplete. Death from loss of blood.
			1	347	
			2		
16	100 mg.	$\frac{1}{2}$ cc.	0	221	Normal. Complete absorption.
			1	859	
			2	804	
			3	801	
			4	226	Death resulted.

242 (2765)

The nature of insoluble urease.

By JAMES B. SUMNER and VIOLA A. GRAHAM.

[From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, N. Y.]

Soluble urease is prepared by the authors from the jack bean by the use of two principles: (1) repeated precipitation from slightly acid 30 per cent alcohol by cooling and centrifuging; (2) removal of the two globulins, concanavalin A and B, by al-

lowing them to crystallize out from the solution of urease in dilute aqueous neutral phosphate solution. Urease prepared in this manner has an activity of nearly 30,000 units per gram of dry material. A unit is defined by the authors as the amount of urease capable of producing 1 mg. of ammonia nitrogen from a urea phosphate solution in 5 minutes at 20° C.

Contrary to the previous belief of the authors, it is not possible to obtain urease entirely free from carbohydrate by the above mentioned procedure. A large amount of a pentose gum can be washed out during the precipitation by cooling, but small amounts of the gum appear to be firmly bound to some of the protein present. It is possible also that soluble urease is contaminated with traces of the third jack bean globulin, canavalin, although canavalin is insoluble in acid 30 per cent alcohol, the solvent used for extracting urease.

The authors have been able to free urease from the last traces of pentose gum, and also from any canavalin that might be present, by means of a third new principle, the conversion of urease into an insoluble, though still active form. This is effected by adding small amounts of sodium chloride to neutral 30 per cent alcohol urease, and allowing the solution to stand in a cool place for one or two days. The alcohol and sodium chloride are not added until after the crystallizable globulins have been removed and the material has been filtered. The precipitate of insoluble urease is centrifuged off and washed several times with aqueous neutral phosphate solution.

Insoluble urease is not as active, weight for weight, as soluble urease, but the authors believe it to be purer than the latter. When digested by trypsin its activity is considerably increased, and in a few instances has been observed to be more than doubled. Further action of trypsin leads rapidly to inactivation.

In the table below are given the percentages of cystine, tyro-

	Canavalin	Concana- valin A	Concana- valin B	Soluble Urease	Insoluble Urease
Ash per cent.....	0.13	0.67	0.22	trace	4.3
Percentages on ash-free basis					
Total "N"	15.97	15.74	15.79	14.18	15.33
Cystine	1.0	0.4	3.23	+	1.2
Tyrosin	5.5	5.17	9.44	4.4	4.94
Tryptophan	0.24	2.25	2.34	0.9	1.46
Urease units per gram				28,000	20,000

sin and tryptophan in the three jack bean globulins, and in preparations of soluble and insoluble urease. The methods of Folin and Looney¹ have been used because they permit the use of very small quantities of material. The value for cystine in soluble urease could not be obtained on account of the interfering action of the pentose gum present in this preparation. It is interesting to note that insoluble urease contains a slightly greater percentage of total nitrogen and of tyrosin than soluble urease, and that both of these extremely active enzyme preparations appear to be protein. We are unable to say why the percentage of tryptophan is so much larger in insoluble than in soluble urease. This may have been due to a loss of tryptophan caused by condensation with the pentose gum present in the soluble urease during the hydrolysis with barium hydroxide.

The amino acid percentages of the three jack bean globulins indicate, although they do not prove the matter conclusively, that both soluble and insoluble urease consist of a protein which is not identical with canavalin, concanavalin A, or concanavalin B. The three jack bean globulins differ from the urease preparations also with respect to such physical properties as solubility, crystalline form and color.

¹ Folin, O., and Looney, J. M., *J. Biol. Chem.*, 1922, li, 421-434.

IOWA BRANCH

University of Iowa, April 29, 1925.

243 (2766)

The action of adrenalin, pituitrin and acetyl-cholin on the coronary arteries of the rabbit.

By FRED M. SMITH, G. H. MILLER and V. C. GRABER.

[From the Departments of Internal Medicine, Physiology and Pharmacology, State University of Iowa, Iowa City.]

The present investigation is a preliminary study of the action of the sympathetic and vagus nerves on the coronary arteries of the rabbit. The effect of adrenalin and acetyl-cholin on the coronary flow of the perfused heart of the rabbit was studied because of the action that these drugs are said to have on the extrinsic cardiac nerves. Pituitrin was employed for the purpose of comparing smooth muscle stimulation with that of the sympathetic nerve action of adrenalin.

Adrenalin was introduced into the perfusate by injecting it with a hypodermic syringe directly into the tubing at its attachment leading into the aorta. The concentration of adrenalin used was 1-10,000,000, 0.2 cc. of this being injected at a rate which would produce a dilution of approximately 1-200,000,000 in the coronary arteries. The above low concentration was employed to avoid the complicating effects produced by the extreme cardiac stimulation resulting from the higher concentrations that are ordinarily introduced. Adrenalin in this dilution produced a definite decrease in the rate of coronary flow. The decrease in the rate of perfusion varied from 12 to 22.5 per cent. The diminished perfusion rate began immediately after the introduction of the drug, and persisted up to the point of maximum cardiac stimulation. At this point the rate of coronary flow was slightly augmented for a short time. Even with these low concentrations of adrenalin there was a very distinct stimulating effect on the heart, as indicated by the acceleration in heart rate and increase in amplitude of contraction.

Pituitrin, in concentrations of 1-50,000 regularly produced a striking decrease in the rate of perfusion. This change in the perfusion rate persisted for some time after the drug was discontinued. In those experiments in which the cardiac rate was not controlled, the injection of pituitrin was followed by a reduction in heart rate of about 20 beats to the minute. In some instances there was associated with the decrease in heart rate a slight diminution in amplitude of cardiac contractions. In those experiments in which the heart was driven by rhythmically induced break shocks, the decrease in coronary flow was not as striking, and the effect did not persist as long as in the heart with normal mechanism.

Acetyl-cholin was introduced into the coronary arteries in dilutions varying from 100,000 to 200,000 concentrations. The drug in these concentrations greatly reduced the heart rate, and in some instances produced cardiac standstill. Coincident with the reduction in the rate, the amplitude of the cardiac contraction was diminished. With the onset of the above changes in the heart, the perfusion rate was greatly augmented, in some instances amounting to 180 per cent. The slowing of the cardiac rate and the depression in amplitude of contraction were transient. The increase in the rate of perfusion, however, persisted for some time after the above effects on the heart had subsided. The administration of atropin sulphate in a dilution of 1-20,000 eliminated the effects of subsequent doses of acetyl-cholin on heart rate, and in a large measure the reduction in the amplitude of contraction. The atropin, furthermore, eliminated the action of acetyl-cholin on the perfusion rate except for very transient slight increase during the period in which there was some depression in amplitude of contraction. In the earlier experiments in which higher concentrations of acetyl-cholin were employed, the depression action of the drug on the amplitude of contraction was more striking and prolonged. In those instances the depression action on amplitude was not particularly altered by atropin, and the acceleration in the perfusion rate, even though greatly reduced, was still evident.

In those experiments in which the cardiac rate was controlled by rhythmical stimulation, thus eliminating, in a large measure, the depression in the amplitude of contraction, the same action of acetyl-cholin was observed.

244 (2767)

Study of response to continuous intravenous injection of large amounts of glucose.

By J. D. BOYD, H. M. HINES and C. E. LEESE. (Introduced by J. T. McClintock).

[*From the Physiological Laboratory, College of Medicine, State University of Iowa, Iowa City.*]

Realizing that no isolated series of observations, such as blood sugar curves, the degree of glycosuria, the gas exchange nor the tissue analysis, is adequate in itself as a measurement of carbohydrate capacity, an attempt has been made to ascertain the response elicited by intravenous injection of glucose continuously for several hours, by means of simultaneous studies of blood sugar, hemoglobin, pH and CO₂ content of plasma, urine volume and sugar, respiratory quotient and heat production. The method of study differed from that of Woodyatt and others in that more factors were correlated, larger amounts of sugar were administered, and the recovery period was observed for several hours. Unanesthetized dogs on standard diet were used. The method of administration was essentially the same as employed by Woodyatt.¹ Glucose in 30 per cent solution was given at the rate of 4 gm. per kilo per hour. Indirect calorimetry was used to measure gas exchange.

It was found that under similar conditions, the response of the same dog to the standard injection on various occasions was remarkably uniform throughout. Values from different dogs were in fair agreement.

Sugar rapidly left the blood, 80 per cent to 100 per cent disappearing as rapidly as injected. There was usually a peak in the blood sugar concentration curve during the first half hour, after which the divergence was considerable, varying more in different dogs than in the same animal. After injection there was a rapid return to normal limits requiring only ½ to 1 hour. In some cases distinct hypoglycemia ensued.

Hemoglobin fell moderately during the injection, soon returning to normal following its close. Fluctuations in pH were

¹ Woodyatt, Sansum and Wilder, *J. Am. Med. Assn.*, 1915, lxxv, 2067.

slight, never exceeding 0.1, and usually less than 0.05. Carbon dioxide content usually fell.

Urine volume increased markedly during the injection, somewhat exceeding the amount of water injected. During the recovery period it was markedly diminished. Glycosuria appeared coincident with hyperglycemia, and ceased abruptly after injection.

The respiratory quotient rose with the onset of sugar administration, and varied moderately during injection, averaging about 0.96. In a few cases it slightly exceeded 1.0. At a varying interval after injection it fell, in a few cases to values less than the resting level, even though a large amount of the injected glucose remained unoxidized. It will be necessary to consider the fall in the CO_2 content of the plasma in the interpretation of these changes. Heat production, which increased with injection, fell soon after its close, to its initial, or a lower level. The decline was more rapid than that of the respiratory quotient.

Through calculation of the maximum amount of carbohydrate combustion which might have occurred during the experiment, and deducting this and the urinary sugar from the amount injected, it was found that after a 4-hour injection, an average of about seven grams per kilo had been retained by the animal.

The injection did not seem to have any deleterious effects on the animal. There was a rise of 1 to 2 degrees centigrade during, and a quick fall to normal after, the injection. After the experiment the animals would eat their diet, and as a rule the animals receiving numerous injections gradually gained in weight.

245 (2768)

The use of the bicolorimeter for the estimation of the hydrogen-ion concentration of urine.

By VICTOR C. MYERS and LELA E. BOOHER.

[*From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

In connection with a study of the acid-base balance of the blood in alkalosis it seemed necessary to secure information regarding the hydrogen ion concentration of the urine. The use of the bicolorimeter for this purpose was suggested in an earlier communication,¹ but details of the technique remained to be worked out. The method as now employed is outlined below. We believe that it increases the simplicity, delicacy and accuracy of the determination.

Three indicators have been employed—phenol red, brom cresol purple and brom cresol green (or methyl red). Wedges are calibrated for each of these indicators, the range employed for phenol red being pH 6.6 to 8.6; for brom cresol purple pH 5.2 to 7.0; and for brom cresol green (or methyl red) pH 4.6 to 5.4. Phenol red is employed in an 0.02 per cent concentration, while an 0.04 per cent solution is used for the other two indicators. The alkaline wedges employed with the phenol red and brom cresol purple indicators are filled with $m/15$ secondary phosphate diluted with 10 per cent of the indicator solution (1 cc. for 10 cc. of phosphate solution); while the acid wedges are filled with $m/15$ primary phosphate similarly diluted with indicator. The wedges for the brom cresol green indicator are made up with Clark's phthalate-NaOH mixture, the alkaline wedge having a pH of 5.8, and the acid wedge of 4.0. In the calibration of the wedges Clark's phthalate-NaOH mixture is used for the range pH 4.6 to 5.2, and Sörenson's buffer phosphates for the range pH 5.2 to 8.6. Since brom cresol purple covers the range pH 5.2 to 7.0 it will take care of the great majority of urines, although for very strongly acid urines, or for alkaline urines, the other two indicators are necessary.

¹ Myers, V. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1919, xix, 78; *J. Biol. Chem.*, 1922, liv, 675.

For the estimation, urine is collected in clean Pyrex beakers with as little contact with air as possible. Two 1 cc. samples are diluted to 10 cc. with distilled water in Pyrex test tubes. To one of these tubes 1 cc. of the brom cresol purple indicator is added, and the solution mixed. If the pH of the urine falls within the range of the indicator, readings are at once made in the bicolorimeter. If not, 1 cc. of either brom cresol green or phenol red is added to the second tube, depending upon whether the urine is strongly acid or alkaline, and color comparison made. So far as the matching of colors goes, the method has a probable error of \pm pH 0.02 to 0.04.

246 (2769)

Observations on the excretion of an acid urine in alkalosis.

By VICTOR C. MYERS and LELA E. BOOHER.

[*From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

It has been customary to use the reaction of the urine as a guide to the therapeutic administration of sodium bicarbonate. In their most recent discussion of the subject Palmer, Salvesen and Jackson¹ state that the first significant effect on the pH of the urine, *i. e.*, a rise to pH 7.0, can be taken as a safe and reliable guide for discontinuing alkali administration. This is probably true in the great majority of cases, but we believe that occasionally cases are encountered which do not readily excrete alkali, and that in such instances the reaction of the urine is not a safe criterion. We can record observations on two cases where the reaction of the urine remained strongly acid despite the development of an alkalosis. The data are self explanatory.

¹ Palmer, W. W., Salvesen, H., and Jackson, H., Jr., *J. Biol. Chem.*, 1920-21, xlv, 101.

Case	Date 1924	Blood		Urine pH	Remarks
		pH	CO ₂ content		
M. J.	2/26	7.50	63	Less than 5.20	Acute nephritis, receiving small amounts of NaHCO ₃ .
	2/27	7.51	61		
	2/28	7.48	52		
A. W.	4/12	7.55	62	5.21	Laparotomy, receiving NaHCO ₃ .

247 (2770)

Acid-base balance in pregnancy.

By O. H. GAEBLER and G. L. ROSENE.

[From the Departments of Biochemistry and of Obstetrics of the
State University of Iowa Medical School, Iowa City, Ia.]

In recent years considerable discussion has centered around the reaction of the blood during normal pregnancy, since findings in abnormal conditions, notably in the toxemias of pregnancy, must of necessity be compared with the normal. The various observers cited in this abstract all agree that plasma bicarbonate is lowered during pregnancy. Hasselbalch and Gammeltoft¹ confirmed earlier observations of the lowering of carbon dioxide tension of the blood during pregnancy, and Rowe² has recently determined the decrease in alveolar tension of carbon dioxide in a large series of cases.

During the past four months we have carried out plasma pH determinations on a series of thirty-three normal pregnant women. The series includes one to three determinations before delivery and one after delivery. Up to the present, 58 of the determinations before delivery, and 23 following delivery have been completed.

Plasma carbon dioxide content was lower during pregnancy

¹ Hasselbalch, K. A., and Gammeltoft, S. A., *Biochem. Ztschr.*, 1915, lxxviii, 206.

² Rowe, A. W., *J. Biol. Chem.*, 1923, lv, 28.

than during the puerperium in all of the twenty-three cases which have been delivered, the average difference being 8.2 volumes per cent. The pH determinations were done by the method of Myers, Schmitz, and Booher.³ The values before delivery extend over a range from 7.35 to 7.47, the mode lying between 7.38 and 7.42. Those following delivery cover the range from 7.34 to 7.42., the mode being 7.38 to 7.40. Taking into account all variations from 0.01 upwards, the pH was higher before than following delivery in 15 cases, by an average of 0.04; lower before than after delivery in 6 cases, by an average of 0.02; and unchanged in 2 cases. Study of the variations encountered does not as yet convince us that the blood is definitely more alkaline before than after delivery, although the evidence slightly favors that view. The number of cases in which the plasma was more alkaline before than after delivery is over twice that of those which showed the reverse variation; and the average by which the former were more alkaline is also greater than that by which the latter were more acid. The series during pregnancy also extends about 0.04 to the alkaline side of the range which the authors of the method regard as normal, and differs from a series of normals which we are determining in similar manner; while the range during the puerperium corresponds more nearly with the normals. Two points are clear: That the lowering of bicarbonate is fully compensated for by lowered carbon dioxide tension; and that any difference between the pH of the blood in pregnant and non-pregnant normals must be very small. In no case did we encounter values of pH as high as those of Marrack and Boone,⁴ who found values of 7.55 in three cases out of their series of fifteen, and upon this basis postulate the existence of an alkalinæmia during pregnancy.

Hasselbalch and Gammeltoft found the blood more acid before than after delivery, the average difference of pH being 0.05. This difference was entirely removed when lowered alveolar tension of carbon dioxide during pregnancy was taken into account. Subsequently Hasselbalch⁵ calculated a difference even smaller, and one which would have been reversed by the effect of carbon dioxide

³ Myers, V. C., Schmitz, H. W., and Booher, L. E., *J. Biol. Chem.*, 1923, lvii, 209.

⁴ Marrack, J., and Boone, W. B., *Brit. J. Exp. Path.*, 1923, iv, 261.

⁵ Hasselbalch, K. A., *Biochem. Ztschr.*, 1916, lxxviii, 112.

tension. Losee and Van Slyke⁶ interpreted the lowered plasma bicarbonate found by themselves, and lowered alveolar carbon dioxide found by others as indicating increased acid production during pregnancy. Marrack and Boone stress the effect of hyperpnea, also investigated by Hasselbalch and Gammeltoft. Bock⁷ has recently found some evidence for the acid overproduction theory.

Evidence for increased acid production on the one hand, and evidence for a slightly more alkaline reaction of the blood on the other seem rather paradoxical. If increased acid production alone were responsible for the lowered bicarbonate, one would expect lowering of bicarbonate to run slightly ahead of lowering of carbon dioxide tension, pH remaining to the lower part of the normal range. If, on the other hand, bicarbonate were lowered indirectly, through lowering of carbon dioxide (hyperpnea), the reverse changes would be expected. The increased irritability of the respiratory center during pregnancy, found by Hasselbalch, would connect the two factors.

248 (2771)

Physiological action of carnosine.

By J. T. McCLINTOCK and H. M. HINES.

[*From the Department of Physiology, College of Medicine, State University of Iowa, Iowa City, Iowa.*]

The material used in this work was produced by Drs. Louis Baumann and Thorsten Ingwaldsen. The natural carnosine was prepared by them from extracts of horse muscle, and the synthetic was produced by them in their work on the synthesis of carnosine from histidine and β -Alanine. For the method of preparation and the evidence for the chemical purity of the carnosine reference is made to the article by Baumann and Ingwaldsen.¹

⁶ Losee, J. R., and Van Slyke, D. D., *Am. J. M. Sc.*, 1917, cliii, 94.

⁷ Bock, A., *Klin. therap. Wehnschr.*, 1924, iii, 2294.

¹ Baumann, L., and Ingwaldsen, T., *J. Biol. Chem.*, 1918, xxxv, 263.

The action of the natural and synthetic substance was found to be identical in all experiments. When tried separately neither the histidine nor alanine used by Baumann and Ingwaldsen in their synthesis of carnosine gave the physiological action produced by carnosine. Complete hydrolysis under conditions which do not destroy the action of other known substances having a similar action, such as histamine, did destroy the effectiveness of the carnosine. It therefore appears certain that the physiological actions described are due to the peculiarity of the dipeptid, carnosine, and not to any impurities.

Subcutaneous injection of 2 gm. in 11 K. dog caused vomiting, diarrhea and the general symptoms of severe toxic shock. Intravenous injection of 10 mg. in 1½ K. cat under ether anesthesia, gave same result as to intestinal activity and carotid blood pressure fell from a normal of 124 mm. to 94 mm. and on a second injection of 16 mg. it fell from a normal of 109 mm. to 85 mm. or a drop of from 24 to 28 mm. The general systemic effect of the carnosine was similar to if not identical with that produced by histamine, but required larger quantities to be effective. Since our experiments upon intact animals, Goldschmidt² has attempted to show that the action of carnosine upon the circulatory system is entirely limited to the splanchnic area.

Upon the isolated rabbit intestine and also upon strips of guinea pig uterus, carnosine produced a marked increase in the tonal and rhythmical contractions when such strips were immersed in Tyroid's or Ringer's solution containing carnosine in amounts sufficient to give 1-2000 dilution. Its action upon these strips is again qualitatively and quantitatively similar to histamine in dilutions of 1 to 100,000. Koinaroff³ has also shown that carnosine is a powerful stimulant to motor activity of intestines. Further experiments with carnosine to determine definitely the point of action in its effect upon blood pressure and upon involuntary muscle are in progress.

² Goldschmidt, E., *Arch. f. d. ges. Physiol.*, 1924, ccii, 435.

³ Koinaroff, S. A., *Chem. Zentralbl.*, 1922, 636.

PACIFIC COAST BRANCH

Stanford University, April 25, 1925.

249 (2772)

Notes on *Councilmania lafleuri* (Kofoid-Swezy 1921).

By TOYNBEE WIGHT. (Introduced by L. B. Becking).

In June, 1921, Kofoid and Swezy announced a new species of intestinal parasite to which they gave the name of *Councilmania lafleuri*. They attributed to their discovery certain peculiarities found in no other intestinal amoeba, which if confirmed, would prove they were dealing with an entirely new species. They also suggested certain pathogenic properties. A somewhat strenuous denial of the accuracy of their findings was made by certain European protozoologists. By and large, in this country at any rate, *Councilmania lafleuri* has been accepted as a biologic fact.

With abundant material from patients from all over the world, we began an immediate search for the new amoeba. It is the conclusions drawn from the examination of many hundreds of stools and carefully stained preparations of specimens containing cysts and free forms that form the subject of this paper.

For some time previous to the announcement of Kofoid's new amoeba we had been doubtful of the accuracy of Dobell's statement that "the sudden extrusion of clear blade-like pseudopodia—so often seen in *E. histolytica*—is never observed in *E. coli*." (*Amoeba Living in Man*, 1919, p. 78.) As this doubt was in line with Kofoid and Swezy's contention, we carefully followed their method of fixation, *viz.*, hot Schaudinn at 60° C., and observed results.

Since the nearest morphological neighbor to *Councilmania* is *E. coli* let us give Kofoid's own comparison of the two organisms:

FREE STAGES.

Councilmania.

- (1) Very active—pseudopodia thrust out suddenly—ectoplasm sharply differentiated.
- (2) Red cells ingested readily.
- (3) Peripheral chromatin thin layer. Karyosome large eccentric with halo—often seen in premitosis.

E. coli.

- (1) Sluggish—ectoplasm not sharply differentiated.
- (2) Red cells not ingested normally.
- (3) Peripheral chromatin thicker. Karyosome small spherical with halo. Generally eccentric.

It will be observed that the above are differences in degree only, and insufficient in themselves on which to base a ready distinction. With regard to item No. 2, an examination of thousands of free amœbæ of the *Councilmania* type during the last few years has failed to show *one* which contained a red cell. We have tried feeding active forms with fresh blood cells and then examining, but without result. The diet of these amœbæ—as Kofoïd himself shows—is largely bacterial in character. Frequently larger food particles—such as cysts of *Chilomastix*, when these are also present—may be seen included. It would appear from the dietary evidence that *Councilmania* is a fæcal scavenger, and in no sense a true parasite. That it may encircle blood cells occasionally, if free blood be present in the gut, is not improbable, but such unusual diet does not make of it a tissue parasite.

Let us now turn to the more important cystic differences as given by Kofoïd and Swezy:

ENCYSTED STAGES.

<i>Councilmania.</i>	<i>E. coli.</i>
(1) Cyst wall very thick.	(1) Cyst wall thin.
(2) Spheroidal, ellipsoidal or asymmetrical—less often spherical.	(2) Generally spherical.
(3) Less readily stained.	(3) More readily stained.
(4) Glycogen body resistant to Iodine.	(4) Glycogen body stains readily in Iodine.
(5) Nuclei with little peripheral chromatin—slightly eccentric dispersed karyosome.	(5) More peripheral chromatin. Small eccentric massed karyosome.
(6) Chromatoidal bodies less acicular in early stages—massed centrally in later stages and contributing to chromophile buds.	(6) Chromatoid bodies more distinctly acicular—less central massing—no relation to segregation of chromophile cytoplasm.
(7) Chromophile ridge forms bud through pore in cyst wall—which detaches uninucleate amœbæ.	(7) Budding unknown.

Distinctions No. 1 and No. 2 are matters of degree and generalities, but I hope to show these differences in shape and thickness of cyst wall have considerable bearing in this discussion.

Item No. 3 is a mistake. There is no especial difficulty in staining *Councilmania*. Occasionally one strikes a type that is more difficult to stain than the usual run, whether this be *E. nana*, *E. coli*, or *E. histolytica*; and speaking generally the *Councilmania* type are stained as easily as any other of the intestinal organisms.

Item No. 4. There is often seen a retraction of the cytoplasmic contents from some part of the cyst wall. Some stools may show

a majority of the cysts with this peculiarity. There is no suggestion by chemical tests that this is a glycogen vacuole. As a matter of fact, glycogen is rarely seen in cysts of the *Councilmania* type. I attribute this particular vacuolisation to osmotic causes.

Items No. 5 and No. 6 are differences in degree only.

Item No. 7, "Chromophile ridge forms bud through pore in cyst wall which detaches uni-nucleate amoebæ"—This last is the valid basis for naming *Councilmania* as a new species. It is very easy to stain specimens in suitable material showing the ridge. Do not carry decolorization too far and stain somewhat deeply—but it must be noted that the ridge is easily decolorized, far sooner than are the chromidial bodies. By the use of a special stain which shows up the cyst wall distinctly, it may be shown that the chromidial ridge is part of the wall itself. The chromidial ridge in our preparations is not of cytoplasmic origin; nor is it of the same material as the chromidial bodies, for with adequate decolorization it disappears entirely, leaving the chromatoid bodies still heavily stained. There is no connection that we can find between the chromatoid bodies and the ridge. The latter seems to be a fold in the cyst wall and its chromophilic character to partake of the nature of a shadow.

We now come to the "bud" through the "pore" in the cyst wall. Examination of many thousands of cysts in fresh saline smears has failed to show this phenomenon. It does not seem possible that Drs. Kofoed and Swezy can have seen it often, nor do they make the claim to have done so. All their illustrations of this phenomenon are from fixed, stained material. It may be suggested that if they saw this phenomenon in a fresh smear in physiological saline, abnormal physical conditions or some pathological process had brought about the result. If one adds hot Schaudinn to a concentration of cysts, and then examines a smear from the mercurial solution, oftentimes a very large proportion of the cysts will now show protruding "buds"—the "pore" varying from a minute aperture to a frankly ragged tear in the cyst wall.

We have stained many hundreds of such preparations, and reproduced each and all of the wonderful illustrations of Kofoed and Swezy. Again it should be emphasized that before such fixation no such pictures may be seen. After fixation and according to the concentration of cysts, to their degree of irregularity of contour and to the rigidity of their walls, few or many budding

cysts may be seen. Let us now remember the two first distinctive characteristics of *Councilmania* as compared with *E. coli* as given by Kofoed and Swezy, viz, greater thickness of walls and irregularity of contour of the cysts, "often ellipsoidal or asymmetrical." Now, since a non-rigid coherent body tends to assume a spherical shape in a liquid medium, it is obvious that the cyst walls of this type are of a certain rigidity. We have, therefore, favorable conditions for fracture if sufficient pressure be employed. It is suggested that the osmotic pressure of the strong mercurial solution used as fixative may be the cause of the appearances as seen in stained specimens. It is an undeniable fact that measurements of stained cysts are proportionately less than those taken of untreated cysts in a fresh saline smear. It is also true that many cysts after fixation and staining show a radiate structure of quasi-crystalline appearance. The unpublished work of John Field and Dr. L. B. Becking on "Anisotropic and Isotropic Agar" suggests an interpretation of these radiating striæ. Their appearance indicates a condition of stress in the direction of the radius of the cyst. The pressure within and without the cyst must be very great. If a break occurs at a weak point it must be in the direction of one of these radial lines of stress. This internal stress may account for the "bud."

If "budding" were a real and not an artificial process one would expect to find detached amœbulæ. We have never seen these even in those specimens apparently loaded with "budding" forms. The mass of extruded material lies around the neighborhood of the break, and is always in physical union with the cytoplasmic body of the cyst. It is true that we have never made out more than one nucleus in an extrusion—when nuclei are present at all—but there is occasionally seen another nucleus in the act of passing through the "pore" whilst the mass of the preceding cytoplasmic extrusion is still in union with the body of the cyst. Many extrusions—probably the majority—contain no nucleus at all.

In this connection it may be remembered that a few months ago at a meeting of this society there was a beautiful demonstration showing how certain chemical irritants caused an extrusion of the nuclei in pigeon's blood. Frequently one may see the nucleus of a free amœba entirely cast out after hot fixation.

CONCLUSIONS.

It is suggested that there are at least two types of *E. coli*; one—of which a perfect description is given by Kofoed under the name of *Councilmania*—characterized by possessing clear blade-like pseudopodia, well differentiated from the endoplasm, occasionally of exceptional motility, which may be retained even after prolonged centrifugation in cold tap water in the process of concentration. This type seems to have a tendency to the formation of irregularly contoured, thick-walled cysts. Such cysts as these are easily fractured by immersion in a hot mercurial solution such as Schaudinn.

The other type is more sluggish, and has not the clear blade-like pseudopodia in the free state. By concentrating a sufficient number of the cysts of this type and subsequently fixing in hot Schaudinn, some at least of the cysts will probably give the appearances to which Kofoed and Swezy have applied the name *Councilmania*. Whilst Kofoed's *Councilmania* would seem to refer to the first type mentioned, if my reasoning be correct, there is either no such species as *Councilmania* or there is no such organism as *E. coli*. For all organisms that we have been accustomed to include under the term *E. coli* are potential *Councilmania* on the only real distinction made between the two organisms, viz, budding—and this latter phenomenon depends on purely physical attributes and artificial outside causes.

Condition of internal stress produced by osmosis in combination with a rigidity of the cyst wall may be the necessary factors in producing "buds." Whatever be the cause, "budding" forms are not seen in the fresh specimen, or, if they should be, may be ascribed to a pathologic or abnormal physical condition.

Amœbæ of the type described as *Councilmania* are exceedingly common. There is no evidence that they have pathogenic qualities. Their normal diet would class them as fæcal scavengers.

In a stool from which stained specimens of "budding" cysts may be easily made, it is impossible to find any detached "amœbulæ"—which leads one to infer that "budding" is produced artificially in the process of fixation and staining. Many "buds" may be found in direct smears from concentrated cysts after fixation in hot Schaudinn, though none at all are to be seen in fresh saline smears from the same original material.

Budding of amœbulæ in the bowel of the host is contrary to natural reason. It would be suicidal and is therefore impossible,

except as a pathological process. That some such process takes place after ingestion of the cysts by a new host is, of course, possible or even probable.

If "budding" be not a normal process within the bowel, there is no excuse for the naming of a new species. On the other hand, if "budding" be a natural process *E. coli* must be the rarest of intestinal inhabitants and *Councilmania* the commonest.

250 (2773)

Revised formula for the rainbow medium.

By TOYNBEE WIGHT. (Introduced by L. B. Becking).

Since I introduced the Rainbow Medium some three years ago for the study of chemical reactions in the bacterial flora of the gastro-intestinal tract, it has been found possible to simplify the technique of its manufacture to a fool-proof stage.

The formula I now use is:

Distilled water	1000 cc.
Agar	20 gm.
Liebig meat extract	5 gm.
Peptone (Difco)	20 gm.
Normal soda	3 cc.

Boil till agar all dissolved. Add indicators. (Andrade 10 cc. Saturated aqueous Brom-thymol-blue 40 cc. Saturated aqueous Phenol-sulphone-phthalein 20 cc.—The last indicator may be omitted, though if this be done, the higher degrees of alkaline formation may not be readable).

Filter through a sheet of absorbent cotton repeatedly until the temperature of the filtrate has fallen to 55C. Stir filtrate rapidly whilst adding one gram of Basic Lead acetate in 100 cc. of warm distilled water. Simultaneously add in the correct proportions whatever sugars it is desired to use. Repeat filtration through the cotton two or three times. Fill the tubes to the desired depth. Then plug. Sterilise at 10-15 lbs. for twenty minutes. Make long slants—not short as in double Russell tubes. Stab centrally with needle and stroke surface. (Kligler, for a similar medium, advises stabbing close to the side of the tube at the lower edge of the slant, but this method does not give as much information as the central stab).

Prior to the introduction of the Rainbow medium multiple indicators had not been used in solid media. The advantage of solid medium is that the reaction at any part of the tube may be approximately determined. Bronfenbrenner had previously advocated a mixture of China-blue and Rosolic acid for liquid media.

The middle of the *Rainbow* scale (yellow-green) indicates approximately neutrality. Violet is strong alkali, and red strong acid. It will be noticed among other things that sulphide formation takes place in only alkaline or neutral conditions.

There would seem a possibility that some modification of this medium might be of value to the phyto-physiologist and pathologist.

251 (2774)

The identity of the pigments in the purple bacteria.

By L. B. BECKING.

[From the Laboratory for Economic Biology, Stanford University, Calif.]

There exist certain groups of bacteria which possess a peculiar purple color. This color ranges from a pale bluish pink to a deep crimson, almost like Ruthenium red. The bacteria concerned may be grouped according to their physiological behavior into four distinct classes:

Name	Chief authors	Phys. characters	React. medium	Relation to oxygen	Relation to light.
I. Purple sulphur bacteria. Thiorhodie.	Winogradsky	Autotrophic	Basic	Oligo-oxyphilous	Needed
II. Molisch bacteria. Athiorhodie.	Molisch Buder (2)	Heterotrophic. Great carbohydrate consumer	Acid	Oligo-oxyphilous	Needed
III. Brine bacteria.	Peirce	Heterotrophic. Do without sugar	Neutral	Oligo-oxyphilous	Not needed, but not harmful, even in high intensities
IV. "Algal" symbiont of Chloedecton.	Uphof	Autotrophic (?)	Acid (?)	(?)	(?)

The morphological variation is also very great.

The almost algal Chromatium, the pediococcoid Thiopedia, Spirillæ, Bacillæ, and Cocci all possess the pigment purple color.

There is therefore no *a priori* evidence for the identity of the pigments.

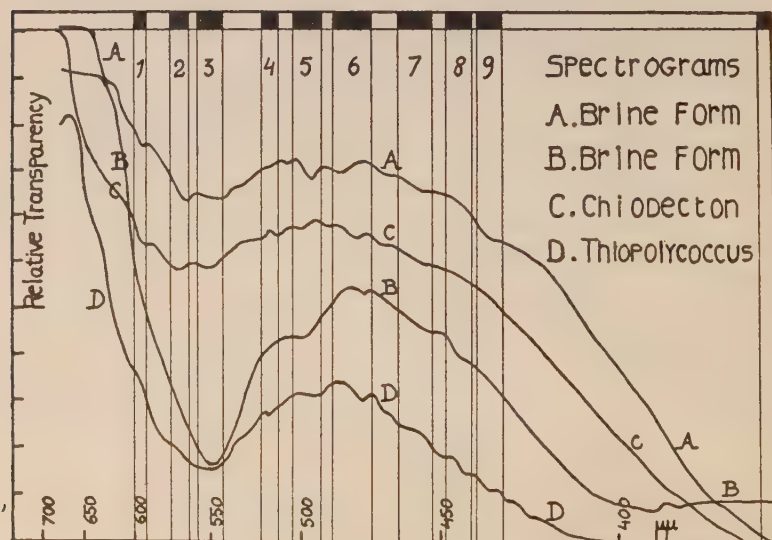
Classes I and II, the true Rhodobacteria, seem to have identical pigments (Molisch). One of them is red, probably a carotinoid, bacterio-erythrin, the other is green, soluble in absolute alcohol and is called bacteriochlorin*. The combination of both pigments is called bacteriopurpurin. Bacteriochlorin has a marked narrow absorption-band with an axis near the sodium lines D.

Bacterio-erythrin is characterized by two distinct absorption bands in the green and bluegreen.

An absolute alcohol extract of Thiopolycoccus yielded a green solution with a bluish green fluorescence. The fluorescence spectrum of this solution will be discussed elsewhere.

The spectra of the following forms were studied.

a. Thiopolycoccus, from a brackish, arsenic-containing, black mud from Owen's lake, California (collected spring 1924 by Dr. F. M. Scott), raspberry color.



*The name bacterioverdin must be reserved for the pigment of the green bacteria. See Buder.¹

The name bacteriorhodin could be used for another red bacterial pigment, such as prodigiosin.

b. A coccoid brine-form from a saturated brine. Owen's lake (collected autumn 1924 by Dr. R. E. Swain), strawberry color.

c. A short bacillus, isolated from a red brine in a Redwood City saltern by Miss D. Burgess (peptone-brine agar), bluish pink.

d. The Florida lichen *Chiodecton* (kindly furnished by Dr. J. C. Th. Uphof, Orlando, Florida), vermilion color.

The foregoing forms were studied with the aid of a micro-spectral ocular of Engelmann, combined with a rotating mirror funnel camera with attached slit and movable plate-holder. This arrangement (which will be described elsewhere) enabled us to photograph the spectra of minute organisms ($5 \times 15\mu$). By the construction of two regulable slits in the ocular (one for the comparison spectrum, the other for the absorption spectrum) we were enabled, with the aid of a Leitz monochromator, to perform visual spectrophotometric observations. This last method was used as a preliminary orientation with the following forms:

a. A freshwater *Lamprocystis*, collected by Dr. A. G. Vestal.

b. *Amoebobacter*, collected in San Francisquito Creek. The brine forms were also studied with this method. The photo-spectrogram showed three bands: (1) 600-583, (2) 560-540, (3) 510-490 $\mu\mu$. Band¹ is very faint in the brine forms. The violet absorption as recorded by various authors could not be found; the extreme violet, up to less than 400 $\mu\mu$ remained clear.

Photospectrograms (Eastman Panchromatic film), with varying slit width, (illuminant Leitz mignon lamp) revealed the presence of another band with a maximum intensity near 460 $\mu\mu$. This is probably the band described by Buder,² on *Rhodobacillus palustris*.

Through the kindness of Dr. P. A. Ross, of the department of Physics, Stanford University, the author was enabled to measure the opacity of the negatives with the aid of an improved Boys' radiomicrometer.

The illuminant was an electric bulb, in series with seven tungsten filament lamps, and ran on a 104 volt battery current. The light remained constant throughout the observations as determined by check readings. By varying the slit width the radiomicrometer mirror could be adjusted on the 100 mark of the scale division. The logarithm of the reciprocal of this number

¹ Buder, J., *Ber. deutsch. Bot. Ges.*, 1913, xxxi, 80.

² Buder, J., *Jahrb. Wiss. Bot.*, 1919, lviii, 525.

—2 had simply to be subtracted from any adjusted reading to obtain a direct measure for the opacity of the negative. The ordinates in the accompanying figure, therefore, represent the relative transparency of the negative. The measurements have no absolute value, inasmuch as several plate factors, such as spectral sensitivity, development, etc. are not taken into account.

The abscissæ are expressed in millimicra. Readings were taken at intervals of .7 millimeters, in other instances at intervals of .35 millimeters. Every curve represents from 150 to 200 measurements. Minima in the curves correspond to absorption maxima. On the steep slopes these minima are difficult to observe, even with the radiomicrometer. Not counting the extreme red and violet absorption, the radiomicrometer measurements show the presence of 9 bands, more or less coincident in the four forms. The following table records their chief peculiarities:

Band.	Range.	Maximum.	Remarks.
1	600-583 $\mu\mu$	590 $\mu\mu$	Bacteriochlorin. Absence in C due to spacing of measurements.
2	577-560	568	Very faint, invisible to naked eye.
3	558-541	548	Shifted in brine bacteria. Chief bacterioerythrin band.
4	518-511	515	Very faint, only recorded by radiomicrometer.
5	507-493	500	Strong band. Bacterioerythrin.
6	489-473	480	Position varies somewhat, visible on photogram.
7	463-452	459	Faint but unmistakable. Recorded by radiomicrometer. Seen by Buder(?)
8	448-444	446	No trace in Uphof's Chiodecton. Recorded by radiomicrometer only.
9	441-433	438	No trace in Uphof's Chiodecton. Recorded by radiomicrometer.
10	400-380	-----	Endabsorption varies considerably with thickness of layer.

Bands 1, 3, and 5 are visible to the naked eye and are recorded by the older authors. The results presented correspond closely to the visual observations of Molisch³. The energy curves are similar to those obtained by Engelmann⁴ with the Vivrodt in-

³ Molisch, H., *die Purpurbakterien*. Jena. 1907.

⁴ Engelmann, T. W., *Bot. Ztg.*, 1888, xlv, 661; Lankester, Ray, *Quart. J. of Micr. sc.*, 1876, xvi, 27; Peirce, G. J., *The Salton Sea*, Carnegie Inst. Publ., 1913, xciii, 49; Uphof, J. C. Th., *Am. J. Bot.*, 1925, xii, 97; Vahle, C., *Centr. f. Bakt.*, 1910, xxv, 178; Warming, E., *Vidensk. Medd. fra. den Naturhist. Forenning i Kjöbenhavn* No. 20-28, 1875; Winogradsky, S., *Bot. Ztg.*, 1887, xlv, 493.

strument except for the wave-length calibration. Buder's results do not agree very well with ours. Satisfactory agreement with most of the older authors (A. Mayer, Engelmann, Warming, and Ray Lankester) has been obtained.

The evidence obtained tends to show a close relation between the pigments of four classes of purple bacteria.

252 (2775)

The influence of acidity in the intestine upon the absorption of calcium salts by the blood.

By LAURENCE IRVING and JOHN FERGUSON. (Introduced by E. G. Martin).

[From the Laboratory of Physiology, Stanford University, Calif.]

If calcium compounds are present in sufficient amounts in the diet, their solubility in the intestinal contents would be a limiting factor for absorption. Apparently calcium chloride and calcium lactate are quite easily absorbed. Salvesen¹ observed that calcium lactate could supply the entire calcium requirement of parathyroidectomized dogs even on a meat diet. Calcium lactate is quite soluble (about 10 per cent) compared with carbonates, phosphates, and most organic salts of calcium.

The solubility of organic calcium salts generally is favored by acidity. It has been suggested² that the acidity of the intestine is a factor in calcium absorption. Inouye³ remarks that lactose feeding favors the maintenance of a sufficiently high blood calcium concentration to prevent tetany in parathyroidectomized dogs, and that this diet favors an acidophile flora. The diffusible calcium salts of milk are increased by acidification, as well as by tryptic digestion.⁴

In order to secure evidence on the absorption of calcium salts from the intestine these experiments were performed. Under

¹ Salvesen, *Acta Medica Scand.*, 1924, lx, suppl. 6, 5-159.

² Orr, Holt, Wilkins, Boone, *Am. J. Dis. Child.*, 1924, xxviii, 574-81.

³ Inouye, *Am. J. Physiol.*, 1924, lxx, 524-37.

⁴ György, *Biochem. Zeit.*, 1923, cxlii 1-10.

anesthesia the intestines of dogs and rabbits were injected with CaCl_2 solutions buffered at several hydrogen ion concentrations. Blood samples were removed at the start and at successive periods, and the serum Ca determined by the method of Kramer and Tisdall.⁵ It has been shown⁶ that serum calcium constitutes practically the entire blood calcium.

CaCl_2 solutions were selected as the most soluble of calcium salts, buffering of the acid solutions being accomplished by citrate mixtures. In general, alkaline buffers are objectionable because of the insolubility of their calcium compounds, but the glycocoll mixture was finally used. Possibly because of digestion, the pH of such a solution was not stable, and moved rapidly toward an acid condition in the intestine.

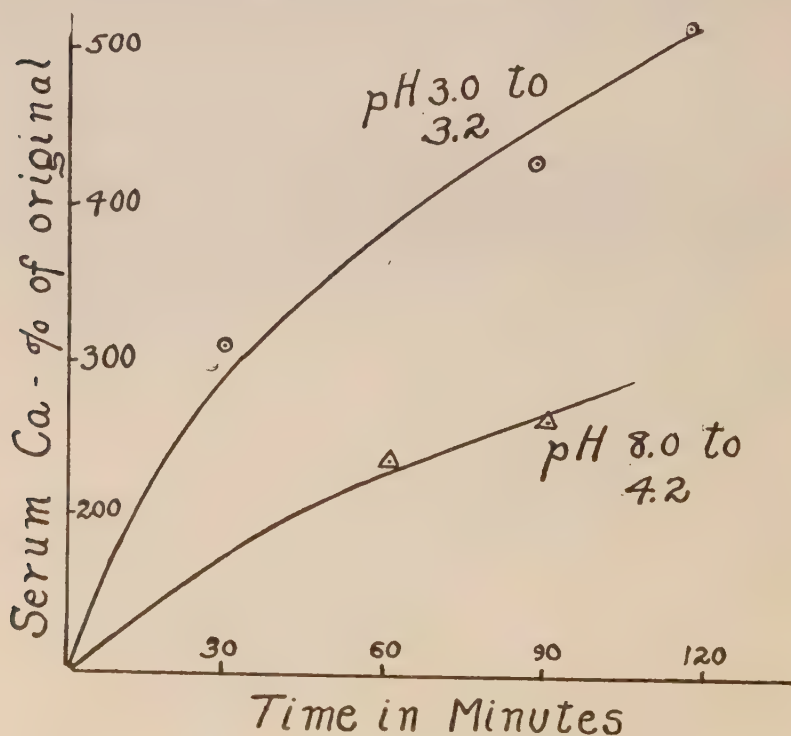


Figure 1.

⁵ Kramer and Tisdall, *Bull. Johns Hopkins Hosp.*, 1921, xxxii, 44-50.

⁶ Howland and Kramer, *Am. J. Dis. Child.*, 1921, xxii, 105-119.

Rabbits did not prove such suitable subjects for the experiments because of the variability found in their normal serum. It is also likely that urethane is not an ideal anesthetic for use in such experiments because of its possible hydrolysis and increase of the alkalinity. All of the experiments, however, were performed on pairs of animals, the treatment differing only in the acidity of the calcium chloride solution. The experiments with dogs, which appear most suggestive, were controlled by blood counts which indicated that no dilution or concentration had occurred sufficient to reverse the order of the findings.

Figure 1 shows the results of experiments with two dogs receiving CaCl_2 solutions buffered at pH 3.0 and 8.0 respectively. There was no question of the rapid absorption of calcium by the serum, and of the fact that absorption from the acid medium was more pronounced.

Figure 2 shows results with another pair, one receiving CaCl_2 solution buffered at pH 3, the other unbuffered neutral CaCl_2 . Here the difference in absorption is not so great, but still favors the more acid solution. These figures represent results typical

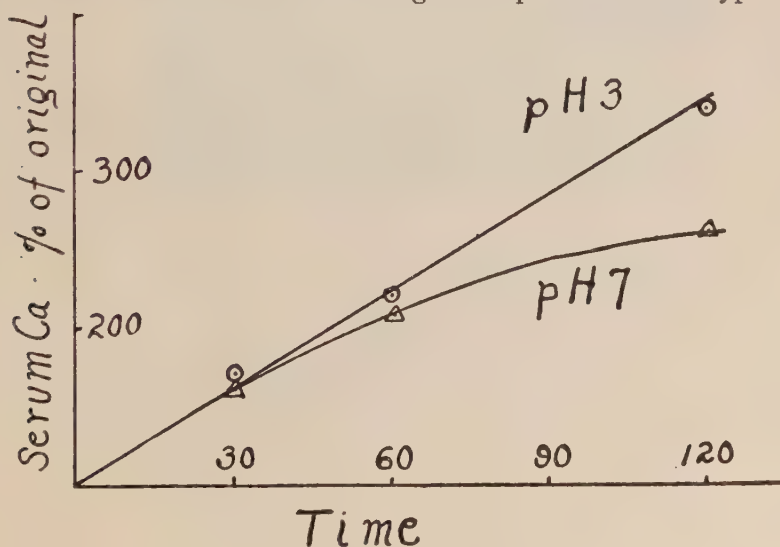


Figure 2.

of those obtained with other dogs and with rabbits, although the rabbits were not so regular.

It is not shown why calcium from CaCl_2 solution should be more readily absorbed from acid than from neutral solutions, as its ionization would be nearly total in either case and its solubility complete. The injected salt, however, would gradually receive contributions from the several sources of intestinal fluids, producing a quite different solution. Calcium salts would be limited in solubility to that of their least soluble combination, and under such conditions an acid buffer would preserve them in solution. The general separation of the curves with increasing time agrees with this idea, for absorption from neutral or alkaline solutions gradually decreases, while from buffered acid solutions it remains about constant.

253 (2776)

The action of strophanthus on the chloralized heart.

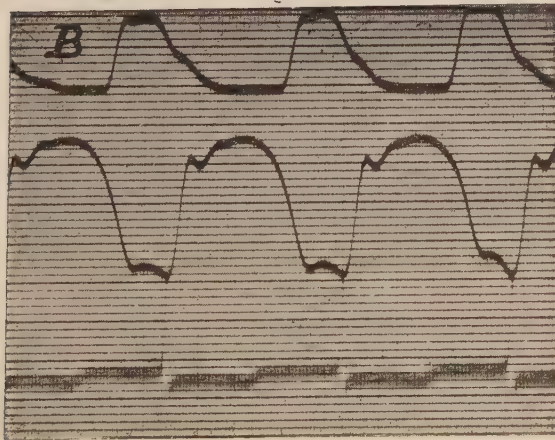
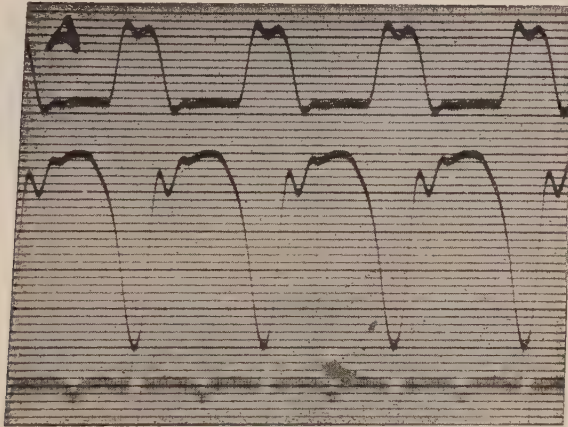
STEPHEN d'IRSAY. (Introduced by Frederick Eberson).

[*From the Departments of Medicine and Physiology, University of California.*]

In a recent paper¹ I was trying to show—for the cold blooded heart—that in chloral hydrate, if applied in appropriate concentrations, we possess a substance which paralyzes the nervous elements of the heart in precedence to the muscular ones. It was suggested there that we might take advantage of this functional interval (elapsing between the elimination of the nervous elements and the paralysis of the sinus: the end stage of chloral action) in order to analyze the point of attack of certain pharmacological agents. The reactions of the digitalis group were tested first, for, although it is generally known that digitalis action is primarily myotropic, there remains a possibility of neurotropic action, particularly concerning chronotropic and dromotropic activities. The experiments have been carried out on the perfused heart of terrapin (Engelmann's method.) The chloral

¹ d'Irsay, S., and Priest, W. S., *Am. J. Physiol.*, 1925, lxxi, 563.

hydrate concentrations used were 0.01-0.025 per cent. With these concentrations, the functional "denervation level" was reached in about 15-35 minutes.¹ The action, with changes in conductivity and latent period as criteria, is reversible, if Ringer's solution is reapplied before the denervation level of these functions is reached; in later stages chloral hydrate creates irreversible conditions. The perfusion in the interval was therefore conducted with the test drug alone. For this strophanthus was used, in concentrations of from 0.1-0.02 per cent, under pressures of 5-10 mm. Hg., at a perfusion rate of 3-5 cc. per min. Optical auricular and ventricular myograms were taken simultaneously with electrograms. Perfusion with chloral hydrate alone and with stro-



phanthus without previous action of chloral served as controls.

The rate decreased both with and without chloral hydrate, after short transitory acceleration, and the heart stopped always in systole. The auricle was the *ultimum moriens*. The conduction time is increased about 80-100 per cent, from 0.4 to 0.7-0.8 sec. This shows that the negative chronotropic and negative dromotropic influences of strophanthus are not dependent upon the functioning of nervous elements. The period of latency, a property depending upon the intactness of nervous activities¹—being prolonged from 0.12 sec. to 0.36 sec. in average, is not affected by strophanthus. The amplitude of contractions is not markedly affected. The tonus, if judged by the sinking of the basal abscissa, is lowered after short rise, as it is in the normal digitalized heart. Other phenomena observed were (1) nodal rhythm, which can be explained by the positive bathmotropic action of strophanthus being coupled with the elimination of positive chronotropic and dromotropic nervous influences; (2) partial diastoles (peristalsis of the heart); (3) Luciani's periods, both in normal and denervated hearts, leading to the assumption that this is a purely muscular phenomenon; (4) distortion (broadening and notching) of the QRS complex of the electrogram, a

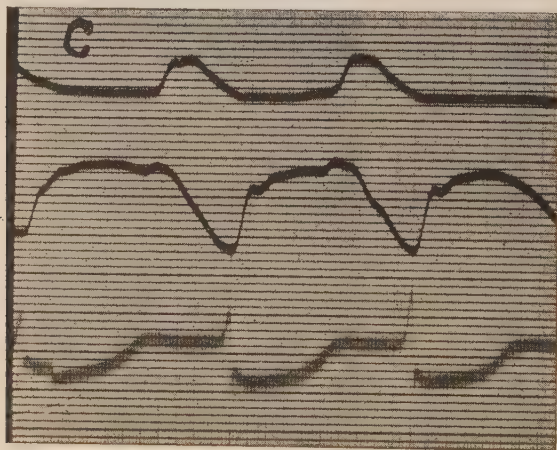


FIG. 1.

- A. Typical experiment. Heart perfused with Ringer's solution.
- B. The "denervation-interval", after perfusion with chloral hydrate.
- C. Perfusion with Strophanthus during the interval. Upper curve: auricle, middle curve, ventricle, lower curve: electrogram. Abscissa: 1 mark: 0.04 sec. Ordinate: 1 mm: 10^{-4} V. Resistance: 8000 Ohms.



FIG. 2.

Nodal rhythm and depressed intraventricular conductivity under strophanthus. Coordinates as above.

sign of depressed intraventricular conductivity. These observations permit us to describe the action of a digitalis body as purely myotropic, having the same effects in the cold blooded heart denervated by chloral hydrate, as in the normal organ. These preliminary statements will be followed up by a report including particularly the bathmotropic and tonotropic reactions of the chloralized heart to digitalis substances.

254 (2777)

Cataphoresis of ultramicroscopic particles in protoplasm.

By C. V. TAYLOR.

[From the Department of Zoology, University of California, Berkeley, Calif.]

The cataphoresis of microscopic granules suspended in the fluid protoplasm of various cells was years ago recorded by several observers (Carlgren,¹ Pearl,² and others).

Recently, I have succeeded in demonstrating the cataphoresis not only of microscopic granules, but also of ultramicroscopic particles in the fluid plasmodia of the slime-mould *Stemonitis elegans*.

¹ Carlgren, *Archiv. f. Anat. u. Physiol.*, 1900, 49.

² Pearl, *Am. J. Physiol.*, 1900, iv, 96.

This Myxomycete is an excellent object for a prolonged study of the structure and behavior of living matter and for experimental observations under highest magnification. It will, moreover, continue its plasmodial stage for months under a bell-jar in the laboratory, if the conditions of moisture, temperature (about 20°C.) and light (free from direct sunlight) are kept reasonably constant.

Cataphoresis of microscopic granules.

If a portion of one of the newly-formed pseudopodia be carefully excised and mounted in a shallow hanging drop on a cover slip, which is then inverted over a moist chamber, with transmitted light one may observe in the protoplasm numerous microscopic granules of various shapes and sizes. Especially the smaller granules exhibit dancing, Brownian movements such as to indicate a distinctly fluid consistency of the protoplasm.

Upon applying a weak direct current (of about 0.02 of an ampere) by means of non-polarizable micro-electrodes, these smaller, oscillating granules gradually migrate toward the anode. If the induced current be very little more than that of 0.02 of an ampere, or if other conditions such as temperature, viscosity and probably additional factors not yet definitely determined be not optimal, migration of the granules may be markedly deterred, or it may not occur.

Cataphoresis of ultramicroscopic particles.

The protoplasm of Myxomycetes may easily be freed from most of its microscopic granules by allowing the plasmodium to migrate through a bit of moist, loosely packed cotton (Lister³). Indeed, granule-free pseudopodia quite readily creep up an inclined cover slip against water dropping from a suspended capillary tube. The coverslip is then carefully removed, with a suitable portion of the plasmodium remaining intact, and inverted over the moist chamber for observation and experiment.

Demonstration of the cataphoresis of ultramicroscopic particles was made with dark-field illumination and by means of an exceedingly weak, direct current (4×10^{-8} amperes) which was applied through especially designed, very minute, non-polarizable

³ Lister, A., 1888. Notes on the plasmodium of *Badhannia utricularis* and *Brefeldia maxima*. *Ann. Bot.*, ii, 1.

micro-electrodes. (Tips of the micro-electrodes were $1\text{--}2\mu$ in diameter).

An 8-ampere arc light, the heat rays of which were screened out by two water filters, was the source of illumination.

The lens system used was that of a modern Leitz mon-objective binocular which included 15X oculars and a 1/7A oil immersion objective, giving a magnification of about 1200 diameters. The dark-field condenser was one recently devised for use with a moist chamber which stands about 5 mm. above the stage of the microscope.

This equipment, when carefully adjusted, reveals in the more or less granule-free, fluid protoplasm of *Stemonitis*, hosts of ultramicroscopic particles displaying exceedingly active Brownian movement.

Upon inserting the micro-electrodes into the protoplasm at points well inside the boundary of the microscopic field, an initial marked effect is usually obvious: Brownian movement of the ultramicroscopic particles ceases, and at times one gets the impression of a clustering of these particles into numerous groups; at any rate, a decided increase in the viscosity of the protoplasm is evident. Within a minute or so, however, Brownian movement reappears, quite as active as before the insertion of the micro-electrodes.

If now an exceedingly weak, direct current (of about 4×10^{-6} amperes) is applied, a most interesting and probably very significant phenomenon soon appears: The ultramicroscopic, dancing particles begin migration. But unlike the microscopic granules which migrated only toward the anode, these ultramicroscopic particles migrate, some toward the anode and some toward the cathode. And if the current be prolonged for some 15-20 seconds, one observes also a considerable group of the particles, still in rapid Brownian movement, remaining about the center of the field. These show no tendency of migration toward either electrode, and so are apparently electrically neutral.

The unique behavior of these ultramicroscopic particles in the protoplasm of *Stemonitis* suggests an interesting analogy in the recent findings of Foster and Schmidt⁴ who were able to isolate the amino-acid histidine from arginine and lysine, depending upon the acidity of the protein hydrolysate, by means of the electric current.

⁴ Foster and Schmidt, *J. Biol. Chem.*, 1923, lvi, 545.

It may be inferred that these particles in the protoplasm of *Stemonitis* which, it will be noted, are apparently of colloidal dimensions, are possibly protein in nature and an integral part of the living substance.

It is of considerable theoretical interest to note also that, since particles of similar dimensions have in recent years been observed in the protoplasm of various cells, it is reasonable to assume that the specific behavior of the colloidal particles in this slime-mold may prove to be of general fundamental importance in further study of the colloidal properties of protoplasm.

255 (2778)

Compensatory hypertrophy of the kidney: The effect of pregnancy and of lactation.

By L. L. MacKAY, E. M. MacKAY, and T. ADDIS.

[*From Stanford University Medical School, San Francisco, Calif.*]

The stimulus to compensatory hypertrophy was given by the removal of one kidney from a rat. The degree of hypertrophy was measured by comparing the weight of the remaining kidney forty days after the operation with the weight of the kidney of rats in which one kidney had been exposed but not removed. This value is expressed as a percentage. Thus if the weight of one kidney of the control rat was 1000 mg. and the weight of the remaining kidney in the nephrectomised rat was 1300 mg., the degree of compensatory hypertrophy was 30 per cent. The percentages given are the averages of groups of rats. It was intended that there should be 25 rats in each group, but since all of the animals did not become pregnant at the expected time this number was not reached in all of the experiments. The diet used was adequate for growth and for reproduction and contained 17.8 per cent of protein, 24.9 per cent of fat, and 42.2 per cent of carbohydrate. The amount of food taken was nearly the same in all experiments except in the lactation experiment. In this instance the caloric intake rose from 25 calories to 90 calories per 100 grams of body weight per day.

The following results were obtained in rats 180 days of age:

	Percentage Hypertrophy
1. Non-pregnant -----	23.9 per cent
2. Pregnant after nephrectomy -----	31.7 per cent
3. Pregnant before nephrectomy -----	32.9 per cent
4. Pregnant and lactating after nephrectomy-----	34.3 per cent

Two experiments were carried out on rats 360 days of age.

1. Non-pregnant -----	24.7 per cent
2. Pregnant after nephrectomy-----	28.5 per cent

Further experiments are now under way which may throw light on the significance of these figures.

256 (2779)

The effect of training on lactic acid excretion.

By J. K. LEWIS, A. W. HEWLETT and G. D. BARNETT.

[*From the Department of Internal Medicine, Stanford Medical School, Calif.*]

To determine the effect of training on the lactic acid excreted in the urine after exercise, an untrained subject began regular exercise twice a week for two weeks, and then daily for three weeks. The exercise consisted in carrying a 30 pound load on a treadmill with 7 inch steps for 5 minutes at a rate of between 80 and 85 steps per minute. Samples of urine were collected immediately before, and for approximately half an hour after exercise. The rate of lactic acid excretion before exercise was determined, and the subsequent excess above this resting rate was attributed to the exercise. The results of these determinations at various periods during the experiment are given in the accompanying table. It shows a definite decrease in the excess of lactic acid as the experiment proceeded. Subjectively this decrease was accompanied by somewhat less distress during exercise and by less fatigue afterward. This decrease might be explained by assuming an increased mechanical efficiency, so that less lactic acid was formed by muscular contraction; or the decrease might be due to a better oxidative removal of lactic acid because of

changes occurring in the muscle itself, or an increased oxygen supply to the muscles from an improved circulation.

Date	Excess of lactic acid.
3-18-25	458
3-20-25	329
4- 2-25	309
4-10-25	199
4-23-25	159
4-24-25	158

257 (2780)

The effect of breathing oxygen-enriched air upon the excretion of lactic acid.

By A. W. HEWLETT, G. D. BARNETT and J. K. LEWIS.

[From the Department of Medicine, Stanford Medical School, Calif.]

Lactic acid was determined in the urine of two subjects before and after a measured exercise. Two groups of four experiments each were made with each subject. In one group the subject breathed air, and in the other oxygen-enriched air containing about 40 per cent of oxygen. In order to eliminate the possible effects of training, the air and oxygen experiments were alternated. The exercise consisted in carrying a 30 pound load for five minutes on a treadmill with 7-inch steps, operated at the rate of 80 to 85 steps per minute, the work performed being about 6000 kpm. in five minutes. The resting rate of excretion of lactic acid bodies in the urine was determined before each exercise period, and the excess excretion above this level during approximately a half-hour period after the exercise. Determinations were made in duplicate by the method of Clausen, using permanganate oxidation as recommended by Long.

Both subjects showed a lower excess excretion of lactic acid when breathing oxygen-enriched air than when breathing air. Average figures for the four experiments in each group are as follows:

Subject.	<i>Excess Excretion of Lactic Acid.</i>	
	Breathing Air.	Breathing 40% Oxygen.
A. W. H.	73 mg.	24 mg.
J. K. L.	324 mg.	198 mg.

We believe the diminution in lactic acid excretion when breathing oxygen-enriched air to be due to lessened lactic acid accumulation in the muscles.

258 (2781)

Effects of cholesterol on smooth muscle of intestine and uterus.

By CLINTON H. THIENES.* (Introduced by P. J. Hanzlik).

* Medical Fellow of the National Research Council.

[*From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco, Calif.*]

In connection with a study of agents and conditions that may alter the responses of smooth muscle toward autonomic drugs, etc., it has been found that cholesterol definitely increases the activity of excised intestines and uteri of cats and rabbits. The results with flaky emulsions, and filtrates from saturated solutions, of the cholesterol in Tyrode's solution at 37° C. were the same. The exact concentration of the cholesterol in solution was unknown, quantitative estimation yielding only traces of the product, but a rough estimate indicated that muscular stimulation was secured with concentrations probably as low as 1:5,000,000. About the only important change conferred on the Tyrode solution by cholesterol was a lowering of surface tension.

The stimulation of intestinal and uterine muscle was essentially the same, being characterized by a prompt increase in the amplitude of contractions without material changes in rate and tonus in the majority of strips (Figure 1). In a small proportion of strips the rate of contraction and tonus were also increased. When the activity of muscles was weak at the start, or reduced by fatigue, it was almost invariably increased upon the direct addition of cholesterol to the bath. Duration of stimulation has been observed for as long as 30 minutes. Since the stimulation per-

sisted after nicotization and atropinization of intestine, and was obtained with uteri whether inhibited or augmented by epinephrine, and was promptly opposed by papaverine, it was due to increase in contractility of the muscle substance independently of nerve endings and ganglia. Apocodeine (intestine) and ergotoxine (uterus), as a rule, abolished or prevented the action of cholesterol; but after these drugs the muscles were poisoned, as they were not responsive to barium. Cholesterol altered the response of the muscles to epinephrine since it prevented or reduced the inhibiting effects of epinephrine on intestine or uterus, and augmented the stimulant action on uterus (Figure 2). Thus far, the responses to other autonomic poisons have been variable or not demonstrably altered. Whether the stimulation is concerned with surface or other effects has not been ascertained, but soap and camphor which exert analogous surface effects give somewhat similar effects in some directions, though not in others, and

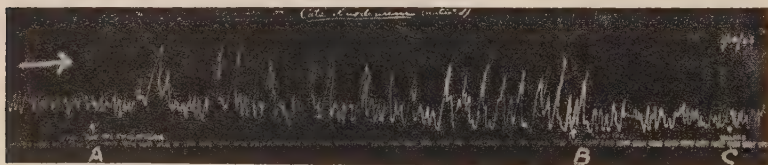


FIG. 1.

Cholesterol on strip of untreated cat's duodenum in 50 cc. Tyrode's Solution at 38° C. At "A", stimulation by 1 cc. of Cholesterol (saturated solution in Tyrode); at "B", partial antagonism by 1 cc. of 0.1 per cent atropine and at "C" washed. Reduced to one-thirtieth.

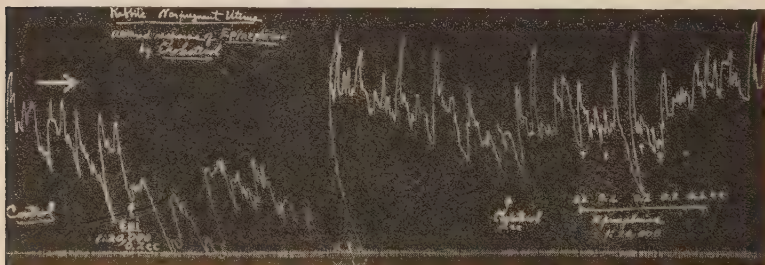


FIG. 2.

Strip of non-pregnant rabbit's uterus in 50 cc. Tyrode's Solution at 38° C. Epinephrine caused depression in Tyrode alone (control) and stimulation after cholesterol (altered response). Reduced to one-twentieth.

themselves cause interesting alterations of smooth muscle responses to certain autonomic poisons.

The results obtained on smooth muscle of the intestine and uterus agree with the older observations of Danilewsky¹ on cardiac muscle of perfused frog hearts which responded to cholesterol with increased systolic contractions without appreciable changes in rate and tonus. They are also in line with vasoconstriction (tonus increase) from cholesterol in perfused organs recently reported by Handovsky,² who attaches importance to this action of the product in certain serum effects. These various augmentations of muscular activity by cholesterol together with other reputed effects on vessels, on metabolism, in pathological states, etc., indicate that cholesterol is a physiologically active product. The study is being continued.

259 (2782)

The anaerobic bacteria of the oral cavity.

By IVAN C. HALL and BEATRICE HOWITT.

[From the California Storatological Research Group,* University of California, Berkeley, California.]

Investigation of 55 samples of saliva collected from 43 individuals showed, 1st, that sporulating anaerobes are not commonly found; 2nd, that when they are found it is usually impossible to detect the same species in subsequent samples from the same mouth.

Only 6 specimens yielded sporulating anaerobes, which included *B. Welchii*, *B. bifermentans*, an unidentified non-pathogen, *B. tetanomorphus*, and a strongly toxicogenic *B. tetani*. This is believed to be the first record, with rigid proof, for *B. tetani* from the mouth. No importance is to be attached, however, to the occasional presence of any of these micro-organisms in the oral

¹ Danilewsky, *Arch. f. d. ges. Physiol.*, 1907, cxx, 181.

² Handovsky, *Klin. Wchnschr.*, 1924, xxx, 3.

* Supported in part by the Carnegie Corporation, by the American Dental Association and by the Associated Radiograph Laboratories of San Francisco.

cavity; all attempts to prove their continued residence there failed. There was likewise no correlation of these findings with dental conditions.

Aerobic spores were also encountered in 16 samples, but not identified owing to the present unsatisfactory status of their taxonomy. Both aerobic and anaerobic sporulating bacteria are evidently transient saprophytes in the mouth, and there is no evidence that either group has any direct pathologic significance in relation to pyorrhea. Certain species, however, may participate in the deposition of salivary calculus and so indirectly contribute to predisposing irritations. Opinion is reserved as to their responsibility for pulp decay in dental caries.

More importance is attached to the non-sporulating anaerobes of the mouth. While we have not failed to appreciate the numerical frequency of spiral and fusiform organisms in saliva through the use of the dark field microscope, these did not appear in our cultures.

Our present emphasis is upon the almost universal occurrence of a minute, Gram-negative, non-sporulating, gas-forming obligate anaerobe of which we have isolated 24 strains from 35 samples collected from 23 persons. This organism is mainly responsible for the abundant gas almost invariably seen in primary brain-medium cultures from unheated saliva. It is identical with the so-called *Staphylococcus parvulus*, (Veillon), recently recovered by Holman and Krock,¹ which they thought resembled *B. pneumosintes* (Olisky and Gates) in filterability. However, after studying two of their strains and 22 of our own, we disagree both as to its nomenclature and its ability to pass through carefully controlled Mandler filters.

This germ differs from *Staph. parvulus* in failing to produce any fetid odor and in producing abundant gas. It was first discovered by Lewkowicz,² working with Veillon in Cracow in 1901, in the mouth of an 8 day old baby, and described under the name *Micrococcus gazogenes alcalescens anaerobius*; in accord with modern scientific usage we suggest *Micrococcus gazogenes* (Lewkowicz).

M. gazogenes is a minute diplococcus less than 0.5 μ in length

¹ Holman, W. L., and Krock, F. H., PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 280.

² Lewkowicz, X., Arch. de med. Exper., 1901, xiii, 633.

and breadth, Gram-negative, non-sporulating, non-motile and obligately anaerobic. These properties have caused it to be overlooked in most modern work on the mouth flora.

Deep brain is the best medium for its cultivation; no blackening or digestion occurs with or without added iron. It may be isolated from deep agar colonies, which resemble a buckwheat in form. Blood agar surface colonies are small, round, moist appearing, raised, grayish white and non-hemolytic. Gelatin is neither liquified nor blackened. The only visible change in milk is gas production. Broth cultures become turbid and produce gas. No acid is formed in the presence of glucose, levulose, sucrose, lactose, maltose, inulin, mannitol, glycerol or salicin; all cultures remain neutral or become alkaline. Forty-eight separate tests for filterability were made with totally negative results.

Most of our data failed to indicate any direct pathogenicity for rabbits, guinea pigs or mice. There is some evidence that it reduces the resistance of rabbits toward other pathogenic microbes of which they are carriers, notably *coccidia*.

Ten rabbits were immunized by intravenous injection of live cultures, with production of powerful agglutinins (all over 1-10,000) which distinguish 2 serologic groups, group A containing 22 strains, group B, 2 strains. Holman's cultures fell into group A. But, while normal rabbits may harbor *M. gazogenes*, yet produce no agglutinins except under inoculation, humans not infrequently produce low grade agglutinating serums (1-40) the strongest of which have come from persons free or practically free from *gingival* lesions, suggesting that there may be an immunologic factor relating to this micro-organism in pyorrhea alveolaris.

260 (2783)

Inhibition of the edema of paraphenylenediamine by drugs and relationship of the adrenals.

By MAURICE LANE TAINTER. (Introduced by P. J. Hanzlik).

[*From the Department of Pharmacology, School of Medicine, Stanford University, San Francisco, Calif.*]

In a previous study, the mechanism of edema formation in rabbits and cats receiving paraphenylenediamine was found to be concerned with a marked increase in vascular permeability, independently of nerve connections, in the head and neck regions¹. This report deals with a short summary of results on the nicotine inhibition, and certain other features.

The edema has been prevented in 70 per cent of 27 rabbits and cats by nicotine (5.6 to 26 mg. per kilo subcutaneously, or 1.4 to 14.7 mg. per kilo intravenously in divided doses). In the remainder, it has been retarded or not inhibited at all, for reasons which may be apparent from the results on adrenal relationship. The nicotine dosage employed did not cause paralysis of the sympathetic and parasympathetic ganglia. The inhibition was not concerned with the Gasserian ganglia and any hypothetical peripheral synapses in the edema organs, since the edema occurred after local infiltration with, and application of, nicotine, and also after degeneration of these structures. Nicotine did not inhibit the edema of the perfused head and extremities. Hence, the inhibition appeared to be systemic. It was not concerned with circulatory depression, for a high degree of circulatory efficiency was maintained throughout. Since the continuous intravenous injection of epinephrine and reduced blood flow to the head also inhibit the edema (Tainter and Hanzlik¹), as does continued stimulation of the cervical sympathetic nerves (Gibbs), a similar mechanism, namely, vasoconstriction, resulting in reduced blood flow to the head, was suggested for the nicotine inhibition. This could occur through the adrenals, that is, by increased epinephrine output of these glands in virtue of the nicotine, a well-known action of this poison.

The dosage of nicotine used increased the output of epine-

¹ Tainter and Hanzlik, *J. Pharmacol. and Exper. Therap.*, 1924, xxiv, 179.

phrine as indicated by the cava pocket method of Stewart and Rogoff² in cats, and by the denervated and atropinized pupil of Meltzer³ in cats and rabbits, the latter method being chiefly employed, and the pupillary response to epinephrine previously determined. Nicotine did not inhibit the edema in adrenalectomized cats, pupilo-dilatation being simultaneously absent. Pupilo-dilatation was marked during all inhibitions of edema in rabbits with adrenals intact, but it was absent in some rabbits that did not respond with nicotine inhibition, apparently due to inadequate output of epinephrine. Nicotine did not inhibit the edema in an ergotized cat whose sympathetic vasoconstrictors were unresponsive to epinephrine, as would be expected, even though ergot had not impaired the epinephrine output. However, in two rabbits with cut and supposedly degenerated sympathetic nerves, nicotine inhibition of the edema still occurred, apparently an inconsistency, providing the receptive substance on which epinephrine acts, was degenerated, but this was not ascertained. Of great importance was the fact that other drugs, which increased the epinephrine output of the adrenals like nicotine, namely, strychnine, picrotoxine and santolin, also inhibited the edema in the majority of rabbits, pupilo-dilatation occurring simultaneously in all those in which this was observed. Hence, these results were strictly consistent with the mechanism of the nicotine inhibition.

Thus, the edema of paraphenylenediamine was inhibited by essentially the same mechanism, the determining factor being presumably a decreased blood flow through the head and neck, however this might be brought about, whether by ligation of vessels, by sympathetic nerve stimulation, by injection of epinephrine, or by drugs which caused an increased output of epinephrine from the adrenals.

² Stewart and Rogoff, *J. Pharmacol. and Exper. Therap.*, 1916, viii, 479.

³ Meltzer, *Am. J. Physiol.*, 1904, xi, 37.

MINNESOTA BRANCH.

University of Minnesota, May 6, 1925.

261 (2784)

Distemper in the silver fox (*Culpes vulpes*).

By R. G. GREEN.

[From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.]

An epidemic of very large proportions has occurred on a number of fox ranches in Minnesota. The specific disease under consideration had been diagnosed as "fox distemper" because of its similarity, from the clinical aspect, to the disease in dogs which is known as "canine distemper." The varied features of canine distemper, which is described as appearing in several forms—the upper respiratory, the central nervous system, and the intestinal types—were all present in the foxes afflicted with this disease on each individual ranch. Most foxes exhibited a very marked infection of the conjunctiva and nasal cavity, from which issued a purulent discharge. A large number of foxes exhibited nervous symptoms before death, ranging from muscular twitching to violent convulsions. A diarrhea appeared to be always present.

Many foxes were not recognized as being sick but were found dead in their pens, although visited daily. Autopsies on a hundred foxes showed a great preponderance of pneumonia, usually extensive. The liver was usually congested; the spleen congested and enlarged. The intestinal mucosa was usually inflamed in the lower portion. Ulcerations were present in the intestine in some cases.

Bacteriological studies of foxes dying on the ranches yielded a great variety of organisms. Streptococci and various diplococci predominated in the pneumonias. *Bacillus bronchosepticus* could be isolated from the nasal secretions. Blood cultures yielded a great variety of organisms. Usually a mixed culture was ob-

tained. Because of the large numbers of organisms encountered, it was believed that these were secondary invaders, and that much of the pathology observed was due to these secondary invaders. It was noted that some few foxes dying on the ranch did not exhibit pneumonia. Some did not exhibit a very marked discharge from the eyes and nose. It was therefore believed that these two pictures did not form a part of the primary infection.

Experiments in transmission of the disease to well foxes were undertaken in order to get rid of the secondary invaders. Those parts of a diseased animal were chosen for this work in which the smallest variety of organisms was found. The disease was successfully transmitted to well foxes by the direct injection of blood, and by injection of emulsified brain and spinal cord.

The diseased foxes used for transmission represented all types of the disease, some with pneumonia and nasal discharge, and some without these findings. In all cases a type of the disease was readily produced in which there was no marked purulent discharge from the eyes and nose, and in which no pneumonia occurred. In the animals dying of the experimental infection the liver was congested and enlarged, and ulcerating lesions of varying grades were present in the intestine.

The disease was successfully transmitted through several series of animals. From the third member of these series a pure culture of a gram negative bacillus belonging to the genus *Salmonella* was isolated. The material containing this organism in pure culture was injected into the fourth member of the series, and the organism again regained in pure culture. The organism was also regained in pure culture in the injected material in the fifth and sixth members of the series, and recovered in pure cultures in both cases.

The organism was isolated in pure culture and was injected into four foxes. The foxes were injected in pairs on the same day, and the respective pairs died on the same day, about eighteen days later. The organism was recovered in pure cultures from various tissues of these four animals.

In the disease produced by the pure culture, symptoms and pathological findings were found which corresponded exactly to those cases occurring on the ranch which did not show signs of secondary invaders. It is therefore established that this organism of the genus *Salmonella* was the cause of the primary disease epi-

demic upon these ranches, and the large variety of pathology encountered was due to the presence of secondary invaders.

When the organism is injected in pure culture intraperitoneally, there are no immediate symptoms. After three or four days the animal begins to drip at the nose as if it had a very acute cold. This excessive secretion lessens after a few days, and the nose becomes dry. The eyes are watery, and after two weeks begin to sink or recede into the head. The animal shows the first marked signs of being ill from sixteen to twenty days after injection, and dies very shortly after the appearance of symptoms. Very marked convulsions may occur before death following injection of the pure culture. The organism can be isolated from every organ and from the meninges. Pneumonia does not occur. The trachea may be inflamed. The liver is enlarged and congested, as is also the spleen. Lesions are found in the gastrointestinal tract which vary from an intense inflammation to definite ulceration and, in some cases, to definite perforating ulcers which may be multiple. The stomach and bladder may also be inflamed. Intense hemorrhages from these ulcerations sometimes occur.

The organism isolated apparently represents a new species of the genus *Salmonella* when classified according to the definitions of the Society of American Bacteriologists. Its identifying characteristics have as yet not been completely worked out.

NOTE—The writer acknowledges his indebtedness to M. B. Hersdorffer, E. T. Dewey, H. O. Halvorson, and G. H. Scott for assistance in carrying out this work.

262 (2785)

Immunization against scarlet fever using sodium ricinoleate as a detoxifying agent.

By W. P. LARSON and WOODARD COLBY.

*[From the Department of Bacteriology and Immunology and
Department of Pediatrics, University of Minnesota,
Minneapolis, Minn.]*

Scarlet toxin, the potency of which was 15,000 skin doses per cc., was treated with sodium ricinoleate so that the final dilution of the soap was 1 per cent and this mixture used to immunize against scarlet fever.

After a few preliminary tests it was found that the toxin had been completely neutralized by the soap, so that 1,000 skin test doses were perfectly safe for children. Early in our work we found that very much larger doses could be given. One hundred forty-eight children, ranging from one to sixteen years in age, with positive Dick tests, were treated with varying amounts of "neutralized" toxin. After having received one treatment of soap-toxin, they were given Dick retests in periods of time ranging from five to twenty-one days. The results are given in the accompanying table.

Dose	No. of Cases	Day of Retest	Percentage of Negative Skin Tests
1000	18	5th	48
1500	31	7th	66 $\frac{2}{3}$
2000	44	8th	68 $\frac{1}{2}$
3000	18	8th	87
4000	21	8th	90
Total	132	21st	96

As may be seen from the above table, 96 per cent of the cases gave a negative Dick reaction three weeks after having received a single injection.

The treatment was followed by a mild local reaction in 15 per cent of the cases. The reaction consisted of a slight reddening and swelling about the area injected, appearing about twelve hours after the injection, and disappearing after thirty-six hours. In no case was a general reaction observed.

A group of nurses who were treated with 2,000 skin test doses experienced no inconvenience whatever from the treatment. There were sixteen in this group. In seventeen days only 37.5 per cent were negative. We believe, therefore, that larger doses are indicated for older children and adults. Since large doses cause the patient no inconvenience, we recommend using 4,000 skin doses, as the larger doses apparently give a higher percentage of immunity than the smaller ones.

The soap-toxin should be allowed to stand at room temperature for four hours before using, to allow it to come to equilibrium. If cloudiness develops upon standing, it should not be used. The soap, as well as the soap-toxin mixtures, must be kept in hard glass containers.

263 (2786)

The effect of concentration upon the neutralization of toxin by sodium ricinoleate.

By W. P. LARSON and H. O. HALVORSON.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

It has previously been shown that sodium ricinoleate possesses the property of detoxifying bacterial toxins.^{1, 2} In the present study an attempt has been made to show the effect of the concentration of both the toxin and the soap upon the efficiency of the detoxification.

One L+ diphtheritic toxin contained in a volume of 0.4 cc. was added to 1.0 cc. samples of a series of soap solutions ranging in concentration from 1 per cent to 9 per cent. After 15 minutes standing, these mixtures were injected into guinea pigs. The results of this experiment are given below:

¹ Larson and Nelson, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278.

² Larson, Evans and Nelson. *Ibid.*, 1924, xxii, 194.

TABLE 1.
Effect of Varying the Concentration of the Soap.

Dose of Toxin	Con. of Soap Used	Total Volume	Result.
L+	1 per cent	1.4 cc.	Died in 3 days
"	2 per cent	"	Died in 11 days
"	3 per cent	"	Died in 28 days
"	4 per cent	"	Paralysis but recovered
"	5 per cent	"	Alive after 78 days
"	6 per cent	"	Alive after 78 days
"	7 per cent	"	Alive after 78 days
"	8 per cent	"	Alive after 78 days
"	9 per cent	"	Alive after 78 days

In this experiment concentrations below 5 per cent did not protect, whereas concentrations of soap of 5 per cent or above did protect. This experiment has been repeated and confirmed. Studies were made in the following experiment to show the effect of changing the concentration of both the toxin and the soap. In these experiments the effect of aging was also studied. To do this a series of samples was prepared, each sample containing an L+ dose for each 1 cc. of 4 per cent soap solution added. One of these was injected without diluting; to the other varying amounts of water were added. One series of guinea pigs was injected after the mixture had stood 15 minutes. Another series was injected after the mixtures had remained in the ice box for 24 hours. The results of the experiment are given in the tables below. The concentrations of soap and toxin given are

TABLE 2.
Effect of Varying the Concentration of Both the Soap and Toxin (Injected after 15 Minutes Standing).

Con. of Toxin L+ per cc.	Con. of Soap in per cent.	cc. Injected	Result
.715	2.86	1.4	Died in 41 days
.417	1.70	2.4	Lived
.294	1.18	3.4	Died in 3 days
.227	.90	4.4	Died in 2 days

TABLE 3.
Effect of Varying the Concentration of Both the Soap and Toxin (Injected after 24 Hours' Standing).

Con. of Toxin L+ per cc.	Con. of Soap in per cent	cc. injected	Result
.715	2.86	1.4	Died in 30 days
.417	1.70	2.4	Lived
.294	1.18	3.4	Died in 2 days
.227	.90	4.4	Died in 2 days

the actual concentrations of the final mixture. A sufficient volume was injected in each case to represent one L+ dose of toxin.

This experiment has been confirmed several times.

This experiment clearly demonstrates the fact that the concentration of both the soap and the toxin is of fundamental importance, of more importance than the actual quantity of toxin injected. It also clearly shows that equilibrium must be reached in a relatively short time, since both sets show about the same results.

Other experiments have been performed in which it was shown that the action of the soap upon the toxin is a reversible one in which the toxin is not destroyed. This was done by preparing a mixture of soap and toxin that would protect, so that when the guinea pig was injected it showed no ill effects. After this mixture had stood for 24 hours, long enough to establish an equilibrium, the equilibrium could be disturbed by adding a quantity of salt solution, as evidenced by the fact that it would now no longer protect.

These experiments tend to show that the neutralization of toxin by sodium ricinoleate is an adsorption phenomenon.

264 (2787)

Antidiphtheritic immunization using sodium ricinoleate as a detoxifying agent.

By W. P. LARSON, E. W. HANCOCK, and HOWARD EDER.

*[From the Department of Bacteriology and Immunology and
Department of Pediatrics, University of Minnesota,
Minneapolis, Minn.]*

The property of sodium ricinoleate to detoxify bacterial toxins has been pointed out in previous papers.^{1, 2} We have studied this problem further to determine whether it is possible to immunize children and adults against diphtheria with toxin which has been detoxified in this way.

A quantity of toxin was mixed with a 1 per cent solution of

¹ PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278.

² *Ibid.*, 1924, xxii, 194.

sodium ricinoleate, so that each cc. of the mixture contained 0.125 L+ toxin. Immediately after mixing, 1 cc. of the mixture was injected subcutaneously into the arms of a group of laboratory workers and volunteer medical students. In all the cases there developed a local redness with some induration, which persisted for five or six days. There were no general reactions observed. Another group was injected with like amounts of soap toxin which had stood at room temperature for six hours to allow the soap-toxin to come to equilibrium. In this group there were either no local reactions or mild reddening at the point of inoculation. No general reactions were observed. In another group, injected with soap-toxin which had stood at room temperature for twelve hours, there were no local reactions.

One hundred forty-nine cases of children and adults with positive Shick tests have been treated with one dose of soap-toxin. Sixty-nine of these have been retested within six weeks after treatment. Of this group 50.2 per cent gave negative skin reactions. We have frequently found the Shick test to be negative as early as four weeks after inoculation.

We wish to emphasize the importance of using only toxin-soap solutions which are perfectly clear. Injections of cloudy solutions are invariably followed by severe local reactions. This point has been emphasized in an earlier publication in which animal experiments were reported.² It is imperative that the soap solution as well as the soap-toxin mixtures be kept in hard glass containers.

265 (2788)

The preparation of pure sodium ricinoleate.

By H. O. HALVORSON. (Introduced by W. P. Larson).

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

The sodium ricinoleate used by Larson in his work on toxin neutralization has been prepared from commercial castor oil.

Castor oil is a triglyceride of ricinoleic acid. It contains small amounts of stearic, hydroxystearic, and oleic acids. The object

of the purification process is to remove practically all traces of these impurities.

In saponifying the castor oil to obtain the crude ricinoleic acid any standard method may be used. The fatty acid obtained is a brownish, viscous liquid that forms considerable sediment upon standing. After about two weeks' standing the semi-clear oil is decanted from the sediment. This is converted into the sodium soap in an aqueous solution. This solution is made up to about 20 per cent, and to it is added a 10 per cent solution of barium chloride until no more precipitate is formed. The barium ricinoleate is removed by filtration, and dissolved in 95 per cent alcohol. The barium soap recovered from 50 grams of fatty acid will require about one liter of alcohol. The barium ricinoleate is soluble in hot alcohol, whereas the barium stearates, hydroxystearates, and oleates are much less soluble. The solution is cooled to about 50° C. and filtered. Activated charcoal is added to the resulting filtrate, and after heating for about ten minutes it is again filtered. The portion that does not dissolve in the alcohol should be discarded. When this alcoholic solution is cooled to 5° C., the barium ricinoleate crystallizes out almost quantitatively. The solution should be kept at that temperature for a day to allow the crystallization to come to completion. The soap is separated from the alcohol by filtration. The process should be repeated, using 800 cc. of 95 per cent alcohol, redissolving, treating with more charcoal, filtering and allowing to recrystallize.

The pure barium soap is converted into the fatty acid by treating it with a 10 per cent HCl solution. After thorough shaking and six hours' standing, the HCl solution is removed in a separatory funnel. The oil is then treated with a 10 per cent H_2SO_4 solution in order to remove the last traces of barium that may be present. Too strong acids must not be used in this step because they have a tendency to break down the fatty acid. By using HCl the greater part of the barium can be recovered in the form of chloride, which can be used again without purification.

After the sulphuric acid treatment, the fatty acid is clarified by centrifuging to remove the barium sulphate suspended in the oil. The resulting clear oil is then separated from the sediment, weighed and dissolved in enough 95 per cent alcohol to make the solution about 10 per cent. To this is added, with constant stirring, the theoretical equivalent of pure NaOH dissolved in the

minimum amount of water. This solution is filtered until it becomes perfectly clear, and then evaporated down to one-fourth its original volume. This solution is allowed to jell and harden, and the resulting cake is cut into fine chips and dried at 35° C.

Soap made in this way consistently resulted in a pure white product that shows within experimental error 100 per cent of the theoretical iodine number. This product will make a water clear solution which shows no turbidity when kept at 5° C. for long periods of time, even though the concentration is as high as 10 per cent. Soaps that will not stand this test do not detoxify efficiently.¹

The sediment that forms in solutions of impure soaps may be either stearates, hydroxystearates, oleates, or polymerization products of the ricinoleate. Due to the tendency of this chemical to polymerize, the pure fatty acid must never be kept in the form of fatty acid longer than is absolutely necessary.

266 (2789)

A note on the photoactivity of cod liver oil.

By F. W. SCHLUTZ and M. MORSE.

[*From the Department of Pediatrics, University of Minnesota, Minneapolis, Minn.*]

As a preliminary step in the investigation of the relationship between cod liver oil and ultraviolet radiation as antirachitic factors, the experiments of Kugelmass and McQuarrie¹ on the photoactivity of cod liver oil were repeated. In every case negative results were obtained.

An apparatus similar to that described by these investigators was employed. Eastman's Speedway plates, possessing properties similar to Seed's Graflex 60, were used. Since these plates proved to be sensitive to the red light in the room, the whole experiment was carried out in total darkness. The cod liver oil was a sample of Mead and Johnson's, guaranteed as to vitamin activ-

¹ Larson, Evans and Nelson, *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 194.

¹ Kugelmass, F. N., and McQuarrie, I., *Science*, 1924, lx, 272.

ity. The apparatus was so arranged that a slow stream of dry oxygen was passed continuously over the surface of the oil during the exposure.

A twenty-four and sixty-six hour exposure to the oxidizing cod liver oil, made alkaline with 10 per cent potassium hydroxide, produced no darkening of the photographic plate. A seven-day exposure to the oil, untreated by alkali, also produced no effect. These results gave no evidence that cod liver oil, while oxidizing, emits ultraviolet light. That oxidation of the oil plays no part in its action as an antirachitic factor would be expected from the findings of McCollum, Simmonds, Becker, and Shipley² that, when air is bubbled through cod liver oil heated to 100° for 10 to 20 hours, vitamin A is destroyed, but the oil is still as effective as the untreated oil in curing rickets.

267 (2790)

The mercury combining power of deproteinized blood.

By PHILIP S. HENCH and MARTHA ALDRICH

[From the Division of Medicine, Mayo Clinic and the Mayo Foundation, Rochester, Minnesota.]

In the study of renal function, simple methods that give clinically accurate data are desirable, particularly to the physician to whom the more complicated blood analyses are not available. The salivary urea index, recently described,¹ is such a method. This test is based on the principle that urea is present in the saliva in definite concentrations depending on the amount in the blood. The salivary urea increases with urea retention in the body. The degree of urea retention can be estimated clinically by the simple measurement of the mercury combining power of the saliva quite as satisfactorily as by the more elaborate blood urea determination.

Since our study of the mercury combining power of saliva and the development of the salivary urea index, we have modified the

² McCollum, E. V., Simmonds, N., Becker, J. E., and Shipley, P. G., *Bull. Johns Hopkins Hosp.*, 1922, xxxi, 221.

¹ Hench, P. S., and Aldrich, Martha, *J. Am. Med. Assn.*, 1923, lxxxi, 1997.

technic so that the same simple test may be applied directly to the blood. An index of the non-protein nitrogen and the urea of the blood is thus easily and quite rapidly available.

TECHNIC.

To 10 per cent trichloroacetic acid in a centrifuge tube is added an equal amount of oxyalted whole blood, preferably 5 cc. of each, and this is centrifuged for four to five minutes. If a centrifuge is not available, the mixture may be filtered, but it may be necessary to use 6 or 7 cc. of blood to obtain sufficient filtrate for the test. On this protein-free filtrate (preferably 5 cc.) is performed the test for the mercury combining power with 5 per cent mercuric chlorid, identically as described for the estimation of the salivary urea index. That is, to the filtrate is added by titration 5 per cent mercuric chlorid until there is a slight excess, as indicated by the appearance of the first faint tinge of brown within three seconds when a test drop is added to a drop of saturated sodium carbonate on a spot-plate.

This procedure may be done with the apparatus² used to estimate the salivary urea index (in which case the figures on the index tube should be multiplied by two), or it may be conveniently carried out in the common graduated centrifuge tube. To the filtrate, mercuric chlorid is added until the end point is reached, and the amount used is read directly from the graduations of the tube. The titration value of the 5 cc. filtrate is multiplied by forty to obtain the mercury combining power of 100 cc. of deproteinized blood.

APPLICATION OF THE TEST

The test was applied to blood obtained from dogs in whom experimental uremia was induced by the administration of urea and by ureteral ligation. It was also performed on samples of human blood. There was throughout a definite parallelism between the mercury combining power of the blood and the non-protein nitrogen substances, chiefly urea. The normal mercury combining power of deproteinized blood is from 70 to 100 cc. of 5 per cent mercuric chlorid. When the nonprotein nitrogen or the urea of the blood was elevated, the mercury combining power also rose. For example, in a sample of blood in which there was

² Hench, P. S., and Aldrich, Martha, *J. Am. Med. Assn.*, 1924, lxxxii, 1194.

480 mg. urea for each 100 cc. the mercury combining power was 500 cc.

From this test, which may be made in fifteen minutes, with a minimal laboratory equipment, may be determined accurately the absence or presence of nitrogenous retention in the body, and the degree of such retention.

268 (2791)

A smoke precipitator used in iodine analysis of food stuffs.

By J. F. McCLENDON.

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

Owing to high potentials needed for the Cottrell Precipitator, and the danger of short circuiting when manipulating the rest of the apparatus, it was decided to try another method of smoke precipitation in the combustion of large samples of food stuffs in the combustion tube, the smoke being passed through an alkaline solution to catch all the iodine. It is very difficult to catch the smoke in wash bottles, in fact it is stated that phosphorus pentoxide dust may be blown through a washbottle without complete precipitation.

The method tried was as follows: The smoke was bubbled through distilled water, this somewhat cooling it and saturating it with moisture at 100°. Its further passage supersaturated it with moisture which caused each smoke particle to be surrounded by a layer of moisture. The precipitation apparatus was a glass tube 7 cm. in diameter and 45 cm. long, closed at one end by fusing the glass, closed at the other end with a rubber stopper, and provided with a side neck 1½ cm. in diameter. By means of holes in the rubber stopper two block tin tubes 1½ cm. in diameter were inserted. One of these tubes was open at the inner end and admitted the hot smoke which passed through the full length of the tin tube (keeping it hot), and then was admitted to the interior of the glass tube, and finally passed out the side neck. The other tin tube was closed at the inner end, and in it was inserted a ½ cm. tube admitting water and keeping it cool. The

smoke in the glass tube was thus between a hot and cool tube. The smoke particles, on cooling sufficiently, were enclosed in droplets of water-vapor which were heated unequally by unequal radiation from the hot and cold tubes. This caused an inequality of the surface tension on the two sides of the drop causing movements of the water surface which, by friction of the air, caused a vortex movement in the air the inertia of which forced the droplet toward the side of the least surface tension, and therefore toward one of the tubes. It is not necessary here to debate the question of which tube, hot or cold, the droplet was precipitated on, it being only necessary at the end of the experiment to wash the outer surface of both the tin tubes and the inner surface of the glass tube to obtain the precipitated smoke.

269 (2792)

Results obtained with the use of the smoke precipitator.

By J. C. HATHAWAY. (Introduced by J. F. McClendon).

[*From the University of Minnesota, Minneapolis, Minn.*]

Heretofore we have experienced some difficulty when precipitating smoke in determining iodine by McClendon's method. The results of smoke precipitation, the theory of which Dr. McClendon has explained, show that the deficiency of the precipitator is in inverse ratio to the amount of smoke given off. When the volume is small the efficiency is around 90 per cent, but when the volume of smoke which passes through is great, it probably does not stop more than one-half of an efficiency of about 50 per cent. Therefore, the average efficiency that has been obtained in all of the determinations would average about 66 per cent.

270 (2793)

The hormone of the parathyroid gland.

By ADOLPH M. HANSON. (Introduced by A. D. Hirschfelder).

[From *Faribault, Minn.*]

The first stage in the extraction of parathyroid active principle is accomplished by extracting in hot acid, preferably *dilute* hydrochloric acid.¹

PREPARATION OF THE HYDROCHLORIC X.

Fresh bovine parathyroid glands (freed from fat as much as possible, without removing parts of the gland) finely divided ----- 30 gm.
Pure distilled water ----- 495 cc.
Concentrated hydrochloric acid (C. P.) ----- 5 cc.

This is boiled for two hours, the solution allowed to cool and made up to 500 cc. with pure distilled water. Most of the insoluble fats are removed by the ordinary process of skimming. Some of the fat adheres to the container in the solidified state. Pyrex glassware is used throughout the entire process, using glass ladles for skimming. The solution is filtered through gauze and fine mesh filter-paper. 5 cc. of the Hydrochloric X are equivalent to 0.3 gm. of the fresh gland. If the fresh glands are finely ground in a Latapie grinder, boiling for fifteen minutes is sufficient to bring out the full amount of the active principle obtainable. Boiling for two hours, however, does not lessen its activity.

The Hydrochloric X may be modified as the first step in its purification, by adding sodium hydroxide, preferably in a concentrated solution to avoid *too* great an increase in its bulk, to the point of maximum precipitation, filtering and concentrating by evaporating in a vacuum.

The injection of amounts of this preparation equivalent to 0.3 gm. of the fresh gland, causes a marked rise in the calcium content of the blood serum of thyroparathyroidectomized dogs, ranging in weight from 9 kg. to 14 kg. In the dogs reported, a rise in the blood serum calcium above the content normal for each dog,

¹ Hanson, *The Military Surgeon*, April, 1923; January, 1924.

or the content found previous to operation, the percentage increase ranged from 27.6 to 80.8; while the percentage increase above the lower level, following thyroparathyroidectomy, ranged from 82.5 to 136.

From the work of Dr. A. M. Hjort, whose article is now in press, it is shown that the Hydrochloric X is very stable, retaining its potency for 15 months, when kept in an ice-box. There is some evidence that points to a lesser degree of stability in neutral or slightly alkaline solution. The author calls attention to the variability of the response of different dogs to the same dose, as well as in the same dog at different times. This renders an absolute standardization difficult by the method of calcium determination. There are too many variables to justify an explanation of these differences in response. Operated dogs are more responsive than normal dogs. Operated dogs, before the onset of tetanic symptoms, respond more readily than do dogs in active tetany. In active tetany, larger doses are necessary to restore the dog to normal. Dr. Hjort also finds that the active element is not in the lipoids of the gland, for, parathyroid tissue, thoroughly dessicated and defatted with acetone and chloroform, yields a very active extract upon boiling with dilute hydrochloric acid. Neutral and alkaline aqueous and alcoholic extracts have so far proven inactive. Acid aqueous and alcoholic extracts contain the active element. He has succeeded in raising the calcium content of normal dogs 100 per cent with a preparation similar to the Hydrochloric X.

Dr. J. C. Collip has recently prepared an extract similar to the Hydrochloric X in that it is hydrochloric acid extract. He reports that it is effective in raising the calcium content of the blood.

An acid extract of the bovine parathyroid, as the Hydrochloric X, or its modification, contains that parathyroid element that produces a pronounced increase in the calcium content of the blood. This is conclusive, as this pronounced rise in the blood serum calcium was secured in thyroparathyroidectomized dogs on a meat diet.

PEKING BRANCH

Peking Union Medical College, April 2, 1925.

271 (2794)

Studies in immunity to tetanus bacilli.

By CARL TENBROECK and JOHANNES H. BAUER.

[*From the Department of Pathology, Peking Union Medical College, Peking, China.*]

So much emphasis has been placed on the part played by antitoxin in immunity that when we found this antibody in the sera of guinea pigs carrying tetanus bacilli in their intestinal tract, we assumed that those animals would be immune to all types of this organism. We felt all the more secure in this assumption since Tulloch¹ has shown that the toxin produced by one type of tetanus is the same as that produced by any of the other types in that they are all neutralized by one antitoxin. When put to a test we found that this assumption is not entirely correct, as is shown by the results outlined below.

We were not satisfied with the methods used by others for the production of tetanus as the incubation period was too short and the animals usually died within two or three days after inoculation. After considerable search we have adopted aleuronot as an irritating agent, when injected with a small number of spores, as symptoms appear on the fifth day, and death follows on the seventh or eighth day after inoculation.

All control animals as well as those used for testing the protective power of sera were bled, and their serum was shown to be free from antitoxin. In addition cultures of their feces were negative for tetanus-like bacilli.

As has already been pointed out² guinea pigs that carry one type of tetanus bacilli for about five months show appreciable amounts of antitoxin in their sera. When tested it is found that these animals are immune only to the type of bacilli which they

¹ Tulloch, W. J., *J. Hygiene*, 1920, xviii, 103.

² Ten Broeck, C., and Bauer, J. H., *Proc. Soc. Exp. Biol. and Med.*, 1924, xxi, 267.

are carrying. When injected with any other types they die in the same length of time as the controls. On the other hand, guinea pigs fed all types of spores are immune to the three types with which we have been working, and it is probable that they are immune to the other three types.

We have produced type serum in rabbits, one set of animals being given repeated injections of washed spores, and the other repeated injections of washed bacilli from young, actively growing, cultures. We refer to these sera as anti-spore and anti-bacterial respectively. Some sera contained antitoxin, while others did not; and we were fortunate in securing for each type of bacillus an anti-spore and an anti-bacterial serum practically free from antitoxin. The anti-spore sera were free from agglutinins, while the anti-bacterial sera contained agglutinins for the type of bacillus injected. One-tenth cubic centimeter of either the anti-spore or the anti-bacterial serum injected intraperitoneally one hour before the introduction of tetanus spores into the muscle, will protect against the type spore used in the immunization, while three cubic centimeters will not, as a rule, protect against the other types. When cross protection occurs it is the anti-bacterial serum which has this property. This cross protection is not very marked as animals injected with three cubic centimeters of serum usually show tetanus, but recover.

When injected before the introduction of spores, the anti-spore serum has a slightly higher protective value than the anti-bacterial; but when injected after the spores, the anti-bacterial has the higher titer. One cubic centimeter of the latter injected three days after the spores are introduced will prevent development of tetanus if the spores are of the same type as those used in the preparation of the serum. In one instance two cubic centimeters injected into each of two guinea pigs four days after the introduction of spores prevented infection, whereas control animals had spasms on the fifth day, and were dead on the seventh. One cubic centimeter of anti-spore serum injected two days after the spores will protect but if injected on the third day there is slight protection, if any.

Guinea pigs that have been injected with a protecting serum followed by spores and aleuronot, and that have shown no tetanus or a tetanus from which they have recovered, are immune to the type used in the first inoculation, but not the other types. Ani-

mals that have recovered from a severe generalized tetanus show practically no antitoxin in their serums, but are immune to the type of organism that produced the primary infection. They are not immune to the other types.

Our results show that antitoxin plays little part in immunity to tetanus, but that there is another immune body or bodies *specific for type* which prevents infection. As yet we have not attempted to determine the nature of these anti-bodies. Tulloch, working with type sera produced by the injection of whole cultures, has shown that they markedly stimulate phagocytosis, and that this property is specific for type. It seems likely that our sera also act by stimulating phagocytosis. It is suggested that serum for the prevention and treatment of tetanus would be of greater value if the animals used for its production were injected with different types of tetanus bacilli in addition to the toxin.

272 (2795)

The hatching phenomena of *Clonorchis* ova.

By O. K. KHAW. (Introduced by E. C. Faust).

[From the Parasitology Laboratory, Peking Union Medical College and from Amoy University, China.]

Ova of *Clonorchis* have a high specific gravity, as they sink in concentrated saline solution within one-half hour, and in pure glycerine (Sp. gr. 1.26) in 48 hours. The shells show markings of an arabesque pattern, best brought out by staining with weak potassium permanganate of toluidine blue. They contain fully developed embryos at the time of discharge from the worm. In spite of these facts, under experimental conditions which may also hold true in nature, they would not hatch out but would degenerate if left undisturbed. Various external factors may operate upon these eggs either singly or in conjunction. The more important of these are as follows:

(a) Mechanical, *e. g.*, pressure of needles in teasing out the eggs; the pressure of cover-glass, or the running in of H₂O under the latter and subsequent streaming with filter-paper. A heavy

pressure short of crushing the embryo, on the cover-glass, will cause the lid to give way and the embryo to be thrown out passively. A light pressure, *e. g.*, touching with the point of a teasing needle, on the other hand, will waken the dormant larva and set its cilia in motion, with the body alternately contracting and extending. Rolling, thrusting and corkscrew movements have been observed, but round-about-turns, as seen in the case of *Schistosoma* larvæ, have never been noticed. The perfectly tight fitting nature of the hard shell would not admit of this. This attempt is often attended with success, but sometimes with failure. In the former case, one side of the operculum would be seen to give way by a forward thrust, aided no doubt by increasing internal pressure, through the opening of which the larva would launch forth, spine foremost, like a torpedo out of a man-of-war.

In nature, the dropping of feces during defecation from a height into the water; the currents carrying the ova to and fro, bringing them into impact with hard objects, and tidal and other washings, if present, may play the rôle of such a mechanical factor.

(b) Physically, the tonicity of the water may help the process of hatching, as evidenced from experiments with saline concentrations. Under natural conditions, the process of concentration, being maximum at water margins of ponds where ova are likely to be deposited, may be brought about by evaporation in the day time. The subsequent dilution by infiltration of tide or of surface water may lead to eggs absorbing the water with consequent popping off of the opercula.

(c) The sudden changes of temperature form another factor. Sudden freezing and thawing, and abrupt raising of temperature from 26° to 36° C. have been shown experimentally to cause spontaneous hatching. This may operate in nature in North China where difference of maximum and minimum temperature is great.

(d) Of the factors working deleteriously on the embryos in the ova, the reaction of the medium is an important one. Alkalinity equivalent to 0.1 per cent NaOH would sterilize the eggs in an hour, as would also acidity corresponding to 0.1 per cent HCl. In alkaline urine and nightsoil mixtures embryonated eggs would perish in two days at room temperature (26° C.) The

fermentative processes found in nature would work in the same way.

Drying is extremely detrimental. A few seconds' exposure would kill the embryos instantaneously. If they were covered with slime, their lives may be a little, though not appreciably prolonged.

Of cheap chemical sterilizers, 5 per cent saturated NaCN would be fatal to embryonated eggs in 90 minutes, and 0.025 saturation of $\text{Ca}(\text{ClO})_2$ would suffice in half that time.

In conclusion, spontaneous hatching takes place under the following experimental conditions: (1) alternate freezing and thawing; (2) sudden raising of temperature, *e. g.*, from 26° C. to 36° C.; and (3) the suitable adjustment of the hypertonicity of the medium, *e. g.*, exposure to 6.25 or 3.15 per cent NaCl for a few minutes, followed by dilution with *aqua dist.* to lower it to isotonicity.

273 (2796)

Abnormal development of the nasal cavity of dogs due to interruption of the respiratory current.

By E. D. CONGDON.

[*From the Department of Anatomy, Peking Union Medical College, Peking, China.*]

Only one experiment on the influence of the respiratory current on the growth of the nose was found in the literature, and this had been incompletely reported. In clinical material the conditions are too complex to bring into sharp contrast the effects of different factors.

The respiratory current was interrupted on the left side of the nose of eleven puppies by introducing cotton through a window cut at the dorsal end of the hard palate. Freshly weaned animals were used, and the experiment continued in most of the series for 46 days. During this period the face is growing rapidly. Packing was changed daily, and the animals maintained in excellent health.

In six dogs the conchæ showed markedly incomplete develop-

ment on the operated side, leaving a wide open space adjoining the septum. The usual marked curvature of the conchæ was almost completely lacking. The septum was slightly concave on the operated side, and the frontal sinus on the average slightly larger. Histologically no difference on the two sides was demonstrated with certainty. The face showed no unusual asymmetry.

A perfect contrast of the condition as to air circulation on opposite sides was prevented by a pressure atrophy of the septum allowing the packing to extend somewhat over onto the unoperated side. This may explain the negative outcome in five of the animals.

Three possible factors in bringing about the arrest of development suggest themselves: namely, infection, stoppage of drainage, and the elimination of the physical effects of the air current, chief among which are fluctuations of pressure, temperature and moisture.

Since mild infection such as observed in the puppies are frequently found on the unoperated side without resultant incomplete development, it is improbable that they have an appreciable influence. The absence of any marked inequality of conchal development in any of ten control animals, some of which must almost certainly have had infections, is further ground for this conclusion. No plug was found which might interfere with the drainage either at the very frequent examinations of animals made during the course of the experiments or at the autopsies. The slight bending of the nasal septum toward the unoperated side suggests a stoppage of drainage, as does also the slightly greater average size of the rudimentary frontal sinuses on the operated side. The evidence as a whole, however, points to the physical effects of the air current as the cause of the abnormal development. A further series of experiments is to be carried out in which it is believed the possibility of a blocking of drainage can be eliminated.

274 (2797)

The effect of repeated administration of ephedrine.

By K. K. CHEN.

[*From the Laboratory of Pharmacology of Peking Union Medical College, Peking, China.*]

In our preliminary report,¹ the results from a rabbit with daily injections of 20 mg. of ephedrine per kilo intravenously for nine days were mentioned. Our present paper deals with a study of the same nature, but much more extensively.

Three series of young, healthy rabbits were selected for experimentation. One series of ten was given intravenous injections of 25 mg. of ephedrine sulphate, irrespective of their body weight, daily for four weeks except Sundays. The total dose amounted to as high as 8.172 times the M. L. D. with reference to the initial body weight, or 817.2 per cent of the M. L. D. Another series of ten was given daily intramuscular injections of 25 mg. of ephedrine sulphate for four weeks except Sundays. Still another series of ten was administered orally 25 mg. of the drug for the same length of time. The total dose in the former exceeded a little the intramuscular M. L. D., while that in the latter was slightly below the oral M. L. D. In all cases with one exception, the body weight was increased, and some animals gained as much as 61 per cent of the initial body weight. Practically all of them were kept alive for about 140 days, and then sacrificed.

Locally, in the intravenous injection, there was development of thrombosis which was gradually absorbed. In the intramuscular administration there was fibrosis at the site of injection, which also disappeared in course of time, for at the end of experiment it was impossible to locate the point of injection. When given by mouth ephedrine did not produce any detectable lesion in the gastro-intestinal tract that could be seen in the postmortem examination.

In these animals, a physiological dose of ephedrine still produced usual effects such as mydriasis, rise in blood pressure and acceleration of heart rate when they were anesthetized. They could still be killed by a M. L. D. The question of habit forma-

¹ Chen, K. K., and Schmidt, Carl F., *J. Pharmacol. and Exper. Therap.*, 1924, xxiv, 339.

tion can apparently be eliminated in so far, at least, as the rabbits are concerned.

At the time when the animals were sacrificed, autopsy was done in every case. Sections were made from lungs, liver, spleen, adrenal body and kidney. While the examination of these sections has not yet been completed, it suffices here to say that no gross pathology can be made out; and that, as far as we have gone, there has not been any demonstrable abnormality in the structures of these visceral organs. There was some cloudy swelling in most of the kidneys, but that was shared by the control animals also.

As ephedrine is a drug that raises blood pressure, it is interesting to study whether there is any development of arteriosclerosis due to hypertension, as many investigators have shown with injection of adrenaline. In one of our several rabbits under anesthesia, an intravenous injection of 0.5 cc. of adrenaline (1-100,000) raised the blood pressure from 96 to 150 mm. Hg., which came back to normal within three minutes; while a similar injection of 25 mg. of ephedrine sulphate (the dose used for daily injection of our animals) in the same animal raised the pressure from 86 to 157 mm. Hg., which did not fall to the former level until twenty-five minutes after. If hypertension were a predisposing factor of arteriosclerosis, we would expect ephedrine to be a more potent drug for the production of such a disease. In fact, it has been reported² that repeated intravenous injections of ephedrine in rabbits caused arteriosclerosis chiefly in the aorta, especially in old or pregnant animals. For the same purpose, we examined in some of our experimental rabbits both grossly and microscopically the heart, aortic arch, thoracic aorta, abdominal aorta, and subclavin, crotid, brachial, cerebral, retinal, splenic, superior mesenteric, inferior mesenteric, renal, femoral, and popliteal arteries. As far as it can be said at present there was only one animal in the series by intravenous injections, none in the other two series, which could be suspected of arteriosclerosis. But it happened that one of our control animals showed the same lesion. So it is quite probable that it was due to spontaneous development.

In a series of white rats, we injected intravenously large doses of ephedrine every other day for seven days. Each dose

² Kiyono, K., and Higashihara, K., *Kyoto Igaku Zasshi*, 1919, xv, 154.

amounted to 74 per cent of M. L. D. Their body weight was constant or somewhat decreased two to three days after the injection, but in all cases it subsequently increased. There were no marked pathological lesions that could be attributed to the effect of the drug.

275 (2798)

The effect of ephedrine on digestive secretions.

By K. K. CHEN.

[*From the Laboratory of Pharmacology and Physiology of Peking Union Medical College, Peking, China.*]

In our preliminary report,¹ a very brief mention was made of the action of ephedrine on the submaxillary gland. In this investigation, the whole system of digestive secretions was studied, including the salivary, gastric, pancreatic, bile and intestinal secretions.

Of nine anesthetized dogs whose Wharton's duct was exposed and cannulated, ephedrine increased the submaxillary flow only in two cases, but had no effect in others. The increase in flow in both animals took place after atropinization when chorda stimulation was ineffective, an evidence that the parasympathetic system has no relation to the action of ephedrine. The dose given did not seem to play any part, for in one animal we injected as much as 40 mg. per kilo in contrast to an ordinary effective dose (1-2 mg. per kilo), and we failed to notice any change in secretion. The increase in submaxillary secretion in dogs by ephedrine is therefore rather an exception than a rule.

In non-anesthetized animals, however, an intravenous injection of large doses of ephedrine usually gives rise to increase of saliva, especially in dogs. Thus in one animal a dose of 25 mg. per kilo produced profuse salivation. When a dose slightly below the M. L. D. was administered, the increase in salivary flow was a constant feature.

¹ Chen, K. K., and Schmidt, Carl F., *J. Pharmacol. and Exper. Therap.*, 1924, **xxiv**, 339.

For gastric secretion, two dogs were operated for Heidenhain stomach pouch, and three for Pavlov pouch. By intravenous injection of ephedrine, there was slight increase of secretion in Pavlov dogs; but by subcutaneous injection of 5-10 mg. per kilo there was definite increase in both the Heidenhain and Pavlov dogs, not only in quantity but also in free and total hydrochloric acid. The increase was not so profuse as with histamine injection, or after a meal.

In connection with the study of the submaxillary secretion in anesthetized dogs, the pancreatic duct and the bile duct were both canulated at the same time. In practically all instances the pancreatic or the bile flow was not changed. We also made a pancreatic fistula in a healthy dog, and a pancreatiko-bile fistula in another. After subcutaneous injection, there was increase in pancreatic secretion in some cases, but that increase was so close to control trials that it is hardly to be accepted as evidence of stimulation.

Two dogs were operated for Thiry-Vella fistula for the collection of the intestinal juice. After subcutaneous injection, the secretion did not start to flow. It is obvious that ephedrine has no action on the intestinal secretion.

LIST OF MEETINGS 1924-1925**Minnesota Branch**

University of Minnesota, Minneapolis, Minn., November 5, 1924, page 183.

University of Minnesota, January 7, 1925, page 307.

University of Minnesota Medical School, March 4, 1925, page 350.

University of Minnesota, April 1, 1925, page 410.

University of Minnesota, May 6, 1925, page 546.

New York

N. Y. Post-Graduate Medical School, October 15, 1924, page 1.

Rockefeller Inst. for Medical Research, November 19, 1924, page 75.

College of Physicians and Surgeons, Columbia, December 17, 1924, page 135.

Cornell University Medical College, January 21, 1925, page 209.

Presbyterian Hospital, February 18, page 267.

Bellevue Hospital Medical College, March 18, 1925, page 315.

College of the City of New York, April 15, 1925, page 363.

Columbia University, May 20, 1925, page 419.

Pacific Coast Branch

Stanford University, June 25, 1924, page 54.

University of California, October 18, 1924, page 118.

Stanford University Medical School, San Francisco, Cal., December 17, 1924, page 256.

University of California Hospital, San Francisco, Cal., February 18, 1925, page 339.

Stanford University, April 25, 1925, page 517.

Peking Branch

Peking Union Medical College, October, 1924, page 199.

Peking Union Medical College, December, 1924, page 261.

University of Peking Medical School, February 25, 1925, page 404.

Peking Union Medical College, April 2, 1925, page 562. •

Western New York Branch

Rochester, New York, October 11, 1924, page 66.

New York Agricultural Experiment Station, Geneva, N. Y., December 13, 1924, page 246.

Syracuse Medical School, April 25, 1925, page 492.

MEMBERS' LIST (Alphabetical)

HONORARY MEMBERS

COUNCILMAN, WILLIAM T.....	Harvard University
REICHERT, EDWARD T.....	University of Pennsylvania
WELCH, WILLIAM H.....	Johns Hopkins University

MEMBERS

ABBOTT, ALEXANDER C.....	University of Pennsylvania
ABEL, JOHN J.....	Johns Hopkins University
ADAMI, J. GEORGE.....	University of Liverpool, England
ADDIS, THOMAS.....	Leland Stanford University
ADLER, HERMAN M.....	Juvenile Psychopathic Institute, Chicago
ALEXANDER, HARRY L.....	Barnes Hospital, St. Louis, Mo.
ALLEN, BENNET M.....	Los Angeles, Calif.
ALLEN, EDGAR.....	University of Missouri
ALSBERG, CARL L.....	Leland Stanford University
ALVAREZ, WALTER C.....	University of California
AMBERG, SAMUEL.....	Mayo Clinic, University of Minnesota
AMOSS, HAROLD L.....	Johns Hopkins Hospital
ANDERSON, JOHN F.....	Rutgers College
ANDERSON, RUDOLPH J.....	N. Y. Agricultural Experiment Station
ASHER, LEON.....	Berne, Switzerland
ASHMAN, RICHARD.....	Tulane University
ATCHLEY, D. W.....	Presbyterian Hospital, N. Y. City
ATWELL, WAYNE J.....	University of Buffalo, N. Y.
AUER, JOHN.....	St. Louis University
AUSTIN, J. HAROLD.....	University of Pennsylvania
AVERY, O. T.....	Rockefeller Institute, N. Y. City
BAEHR, GEORGE.....	Mt. Sinai Hospital, N. Y. City
BAGG, HALSEY J.....	Cornell University Medical College, N. Y. City
BAILEY, C. H.....	Columbia University
BAILEY, CAMERON V.....	N. Y. Post-Graduate Medical School
BAILEY, HAROLD C.....	Cornell University Medical College, N. Y. City
BAITSELL, GEORGE A.....	Yale University
BALDWIN, W. MANNING.....	Albany Medical College
BALLS, A. K.....	University of Pennsylvania
BANTA, A. M.....	Station for Exp. Evolution, Cold Spring, Harbor, N. Y.
BANZHAF, EDWIN J.....	N. Y. Health Department
BARBER, W. HOWARD.....	New York University
BARBOUR, HENRY G.....	University of Louisville
BARDEEN, CHARLES R.....	University of Wisconsin
BARNETT, GEORGE D.....	Leland Stanford University
BARR, DAVID P.....	Washington University
BASS, CHARLES.....	Tulane University

BAUER, J. H.	Peking Union Medical College, China
BAUMAN, LOUIS	Columbia University
BAUMANN, E. J.	Montefiore Hospital, N. Y. City
BAUMBERGER, J. PERCY	Leland Stanford University
BAYNE-JONES, S.	University of Rochester
BAZETT, H. C.	University of Pennsylvania
BECKING, L. B.	Leland Stanford University
BECKWITH, T. D.	University of California
BELL, E. T.	University of Minnesota
BENEDICT, S. R.	Cornell University Medical College, N. Y. City
BERG, WILLIAM N.	Berg Biological Laboratory, N. Y. City
BERGEIM, OLAF	University of Illinois
BERGEY, DAVID H.	University of Pennsylvania
BERNHARD, ADOLPH	Lenox Hill Hospital, N. Y. City
BINGER, CARL A. L.	Hospital of the Rockefeller Institute
BIRKHAUG, KONRAD E.	Johns Hopkins Hospital
BLAKE, F. G.	Yale University
BLAKESLEY, ALBERT F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
BLATHERWICK, NORMAN R.	Potter Metabolic Clinic, Santa Barbara
BLOOR, W. R.	University of Rochester
BOECK, WILLIAM C.	Harvard University
BOLLMAN, JESSE L.	University of Minnesota
BOOTHBY, WALTER M.	University of Minnesota
BOOTS, RALPH H.	St. Lukes Hospital, N. Y. City
BORING, ALICE M.	Peking Union Medical College, China
BOSTROM, ERNEST F.	Geo. Washington University
BREWER, ROBERT N.	Syracuse University
BRIGGS, A. P.	St. Louis University
BRONFENBRENNER, J.	Rockefeller Institute, N. Y. City
BROOKS, CLYDE	University of Alabama
BROOKS, HARLOW	New York University
BROOKS, MATILDA M.	Hygienic Laboratory, Washington, D. C.
BROOKS, S. C.	Hygienic Laboratory, Washington, D. C.
BROWN, E. D.	University of Minnesota
BROWN, J. HOWARD	Johns Hopkins University
BROWN, WADE H.	Rockefeller Institute, N. Y. City
BROWNE, W. W.	College of the City of New York
BULL, C. C.	Johns Hopkins University
BUNTING, C. H.	University of Wisconsin
BURNETT, THEODORE C.	University of California
BURR, HAROLD S.	Yale University
BURROWS, M. T.	Washington University
BURTON-OPITZ, RUSSELL	Lenox Hill Hospital, N. Y. City
BYRNE, JOSEPH	Fordham University
CARLSON, A. J.	University of Chicago
CARPENTER, CHARLES M.	N. Y. State Veterinary College
CALKINS, GARY N.	Columbia University
CANNON, WALTER B.	Harvard Medical School

CARTER, EDWARD P.....	Johns Hopkins Hospital
CASH, JAMES R.....	Peking Union Medical College, China
CAULFIELD, A. H.....	University of Toronto
CECIL, R. L.....	Cornell University Medical School, N. Y. City
CHACE, ARTHUR F.....	N. Y. Post-Graduate Medical School
CHAMBERS, ROBERT.....	Cornell University Medical College, N. Y. City
CHAMBERS, WILLIAM H.....	Cornell Medical College, N. Y. City
CHEN, K. K.....	Peking Union Medical College, China
CHIDESTER, F. E.....	University of West Virginia
CHILD, C. M.....	University of Chicago
CHITTENDEN, R. H.....	Yale University
CHURCHMAN, R. H.....	Cornell University Medical College, N. Y. City
CLARK, GUY W.....	University of California
CLARK, P. F.....	University of Wisconsin
CLOUGH, HARRY.....	University of Rochester
CLOWES, G. H. A.....	Eli Lilly and Co., Indianapolis, Indiana
COCA, A. F.....	Cornell University Medical College, N. Y. City
COHEN, BARNETT.....	U. S. Hygienic Laboratory, Washington, D. C.
COHEN, MARTIN.....	N. Y. Post-Graduate Medical School
COHN, A. E.....	Rockefeller Institute, N. Y. City
COHN, ISADORE.....	New Orleans, La.
COLE, L. J.....	University of Wisconsin
COLE, RUFUS I.....	Rockefeller Institute, N. Y. City
COLE, WILLIAM H.....	Clark University
COLEMAN, WARREN.....	New York University
COLLETT, MARY E.....	Western Reserve University
COLLINS, KATHERINE R.....	Spartanburg General Hospital, South Carolina
COLLIP, J. B.....	University of Alberta
CONGDON, EDGAR D.....	Peking Union Medical College, China
CONKLIN, E. G.....	Princeton University
COOKE, J. V.....	Washington University Medical School
COOMBS, HELEN C.....	New York University
CORI, CARL F.....	State Institute of Malignant Disease, Buffalo, N. Y.
CORNER, GEORGE V.....	University of Rochester
COULTER, CALVIN B.....	Columbia University
COURET, M. J.....	Tulane University
COWAN, GEORGE V.....	Leland Stanford University
COWGILL, GEORGE R.....	Yale University
CRAMPTON, C. WARD.....	N. Y. Post-Graduate Medical School
CRILE, GEORGE W.....	Western Reserve University
CROHN, BURRILL B.....	Mt. Sinai Hospital, N. Y. City
CROLL, HILDA M.....	Yale University
CROZIER, W. J.....	Rutgers College
CRUICKSHANK, E. W. H.....	Peking Union Medical College, China
CSONKA, F. A.....	U. S. Bureau Chemistry, Washington, D. C.
CULLEN, GLENN E.....	University of Pennsylvania
CUMMINS, HAROLD.....	Tulane University
CUNNINGHAM, R. S.....	Johns Hopkins University
CURTIS, MAYNIE R.....	Columbia University
DAKIN, H. D.....	Ossining, N. Y.

DANIELS, AMY L.....	University of Iowa
DANZER, CHARLES S.....	Brooklyn, N. Y.
DAVENPORT, C. B.....	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
DAVIES, H. WHITRIDGE.....	University of Edinburgh
DAVISON, WILBURT C.....	Johns Hopkins University
DAWSON, JAMES A.....	Harvard University
DE BORD, GEORGE G.....	Iowa State College
DE EDS, FLOYD.....	Leland Stanford University
DENIS, WILLEY.....	Tulane University
DETWILER, S. R.....	Harvard University
DEUEL, HARRY J. JR.....	Cornell University Medical College, N. Y. City
DICKSON, E. C.....	Leland Stanford University
DIEUAIDE, FRANCIS R.....	Peking Union Medical College, China
DOAN, CHARLES A.....	Harvard University
DOCHEZ, A. R.....	Presbyterian Hospital, N. Y. City
DOISY, EDWARD A.....	St. Louis University
DOLLEY, DAVID H.....	St. Louis University
DONALDSON, H. H.....	Wistar Institute, Philadelphia
DOOLEY, M. S.....	Syracuse University
DRAGSTEDT, LESTER R.....	Northwestern University
DRAPER, GEORGE W.....	Columbia University
DRAPER, JOHN W.....	New York City
DRESBACH, M.....	Albany Medical College
DUBIN, HARRY E.....	Metz Laboratories, N. Y. City
DUBOIS, E. F.....	Cornell University Medical College, N. Y. City
DUGGAR, B. M.....	Missouri Botanical Gardens
DUNN, HALBERG L.....	Mayo Clinic, Rochester, Minn.
DUNN, MAX S.....	University of California
DUTCHER, R. ADAMS.....	Pennsylvania State College
DUVAL, C. W.....	Tulane University
EBERSON, FREDERICK C.....	University of California
ECKLES, C. H.....	University of Minnesota
EDDY, WALTER H.....	Columbia University
EDMUNDS, C. W.....	University of Michigan
EDWARDS, D. J.....	Cornell University Medical College, N. Y. City
EGGLESTON, CARY.....	Cornell University Medical College, N. Y. City
EGGSTON, ANDREW.....	Manhattan Eye, Ear and Throat Hospital, N. Y. City
EISBERG, HARRY B.....	New York University
EISENBRAY, A. B.....	Western Reserve University
ELSBURG, CHARLES A.....	Mt. Sinai Hospital, N. Y. City
ELSER, W. J.....	Cornell University Medical College, N. Y. City
EMBREY, HARTLEY C.....	Chattanooga, Tenn.
EPSTEIN, A. A.....	Mt. Sinai Hospital, N. Y. City
ERDMANN, RHODA.....	University of Berlin, Germany
ERLANGER, JOSEPH.....	Washington University
EVANS, HERBERT M.....	University of California
EWING, JAMES.....	Cornell University Medical College, N. Y. City
EYSTER, J. A. E.....	University of Wisconsin

FABER, HAROLD K.....	Leland Stanford University
FAHR, GEORGE.....	University of Minnesota
FALK, K. GEORGE.....	Roosevelt Hospital, N. Y. City
FALK, I. S.....	University of Chicago
FALLS, FREDERICK H.....	University of Iowa
FAMULENER, L. W.....	St. Luke's Hospital, N. Y. City
FAUST, ERNEST C.....	Peking Union Medical College, China
FIELD, CYRUS W.....	New York City
FINE, M. S.....	Battle Creek, Mich
FISCHER, ALBERT.....	University of Copenhagen, Denmark
FISCHER, MARTIN H.....	University of Cincinnati
FISH, PIERRE A.....	Cornell University
FITCH, C. P.....	University of Minnesota
FITZGERALD, J. G.....	University of Toronto
FLEISHER, MOYER S.....	St. Louis University
FLEISCHNER, E. C.....	University of California
FLEXNER, SIMON.....	Rockefeller Institute, N. Y. City
FLORENCE, LAURA.....	Rockefeller Institute, Princeton, N. J.
FLOURNOY, THOMAS.....	House of Mercy Hospital, Pittsfield, Mass.
FOSTER, GOODWIN L.....	University of California
FOSTER, NELLIS B.....	New York Hospital, N. Y. City
FRANKEL, FORENCE HULTON.....	Montefiore Hospital, N. Y. City
FRIDERICIA, L. S.....	University of Copenhagen, Denmark
FRIEDMAN, G. A.....	Columbia University
FUNK, CASIMIR.....	State Institute of Hygiene, Poland
GAEBLER, O. H.....	University of Iowa
GAGER, C. STUART.....	Brooklyn Botanical Gardens, N. Y. City
GAMBLE, JAMES L.....	Harvard University
GARBAT, ABRAHAM L.....	Lenox Hill Hospital, N. Y. City
GARREY, WALTER E.....	Tulane University
GATES, FREDERICK L.....	Rockefeller Institute, N. Y. City
GAY, F. P.....	Columbia University
GESELL, ROBERT A.....	University of Michigan
GETTLER, A. O.....	New York University
GEYELIN, HENRY W.....	Presbyterian Hospital, N. Y. City
GIBSON, R. B.....	University of Iowa
GIES, WILLIAM J.....	Columbia University
GITHENS, T. S.....	Mulford Company, Philadelphia, Pa.
GIVENS, MAURICE H.....	Northwestern Yeast Co., Chicago
GLASER, OTTO.....	Amherst College
GOETSCH, EMIL.....	Long Island College Hospital, N. Y. City
GOLDBERG, S. A.....	Cornell University
GOLDFORB, A. J.....	College of the City of New York
GOLDSCHMIDT, SAMUEL.....	University of Pennsylvania
GOULD, HARLEY N.....	Tulane University
GORTNER, R. A.....	University of Minnesota
GRAHAM, EVARTS A.....	Washington University
GRAVES, WILLIAM W.....	St. Louis University

GREEN, ROBERT G.....	University of Minnesota
GREENBERG, DAVID N.....	University of California
GREENWALD, ISIDOR.....	Roosevelt Hospital, N. Y. City
GREGORY, LOUISE H.....	Barnard College, Columbia University
GRIFFITH, FRED R, JR.....	University of Buffalo
GROSS, ERWIN G.....	Yale University
GROSS, LOUIS.....	Brownsville Hospital, N. Y. City
GUENTHER, A. E.....	University of Nebraska
GUTHRIE, C. C.....	University of Pittsburgh
GUTTMACHER, A. F.....	University of Rochester
GUY, RUTH A.....	Feking Union Medical College, China
HADLEY, PHILLIP.....	University of Michigan
HAGAN, WILLIAM ARTHUR.....	Cornell University
HALE, WORTH.....	Harvard Medical School
HALL, IVAN C.....	Cornell University
HALSEY, JOHN.....	New Orleans, La.
HALSEY, ROBERT H.....	N. Y. Post-Graduate Medical School
HAMMETT, F. S.....	Wistar Institute, Philadelphia, Pa.
HANZLIK, P. J.....	Leland Stanford University
HARDESTY, IRVING.....	Tulane University
HARRIS, ISAAC F.....	Tuckahoe, N. Y.
HARRIS, WILLIAM H.....	Tulane University
HARRIS, J. ARTHUR.....	University of Minnesota
HARRISON, R. G.....	Yale University
HARROP, GEORGE A. JR.....	Johns Hopkins University
HARROW, BENJAMIN.....	Columbia University
HARTMAN, CARL G.....	University of Texas
HARTMAN, F. A.....	University of Buffalo
HARTWELL, JOHN A.....	Cornell University Medical College, N. Y. City
HARVEY, E. NEWTON.....	Princeton University
HARVEY, SAMUEL C.....	Yale University
HASTINGS, A. BAIRD.....	Rockefeller Institute, N. Y. City
HATAI, SHINKISHI.....	Tohoku Imperial University, Japan
HATCHER, R. A.....	Cornell University Medical College, N. Y. City
HAWK, P. B.....	Powder Point School, Duxbury, Mass.
HAYDEN, CHARLES E.....	Cornell University
HAYTHORN, SAMUEL R.....	University of Pittsburgh
HEFT, HATTIE L.....	Columbia University
HEIDELBERGER, MICHAEL.....	Rockefeller Institute, N. Y. City
HELMHOLZ, HENRY R.....	University of Minnesota
HENCH, PHILIP S.....	University of Minnesota
HENDERSON, LAWRENCE J.....	Harvard University
HENDRIX, B. M.....	University of Texas
HENRICI, ARTHUR T.....	University of Minnesota
HESS, ALFRED F.....	New York University
HEWLETT, A. W.....	Lane Hospital, San Francisco
HICKERNELL, L. M.....	Syracuse University
HILL, EBAN C.....	Johns Hopkins University

HIRSCHFELDER, ARTHUR.....	University of Minnesota
HOFFMAN, GEORGE L.....	Allegheny County Hospital, Pittsburgh, Pa.
HOLM, GEORGE E.....	Department of Agriculture, Washington, D. C.
HOLMAN, W. L.....	University of Toronto
HOLMES, S. J.....	University of California
HOLT, L. EMMETT, JR.....	Johns Hopkins University
HOOKE, DAVENPORT.....	University of Pittsburgh
HOOKE, SANFORD B.....	Boston University
HOOPER, CHARLES W.....	Brooklyn, N. Y.
HOPKINS, J. GARDNER.....	Columbia University
HOSKINS, R. G.....	Ohio State University
HÖST, H. F.....	Kristiania, Norway
HOWARD, HARVEY J.....	Peking Union Medical College, China
HOWE, PAUL E.....	Department of Agriculture, Washington, D. C.
HOWELL, WILLIAM H.....	Johns Hopkins University
HOWLAND, JOHN.....	Johns Hopkins Hospital
HUBBARD, ROGER S.....	Clifton Springs Sanitarium, N. Y.
HUBER, G. CARL.....	University of Michigan
HUNT, REID.....	Harvard University
HUNTER, ANDREW.....	University of Toronto
HUNTOON, F. M.....	Mulford Co., Glenolden, Pa.
HURWITZ, SAMUEL.....	University of California
JACKSON, C. M.....	University of Minnesota
JACKSON, D. E.....	University of Cincinnati
JACKSON, HOLMES C.....	New York University
JACOBS, WALTER A.....	Rockefeller Institute, N. Y. City
JAFFE, HENRY L.....	Hospital for Joint Diseases, N. Y. City
JEANS, PHILIP C.....	University of Iowa
JENNINGS, H. S.....	Johns Hopkins University
JOBLING, J. W.....	Columbia University
JOHNS, FOSTER M.....	Tulane University
JONAS, LEON.....	University of Pennsylvania
JONES, FREDERICK S.....	Rockefeller Institute, Princeton, N. J.
JORDAN, H. E.....	University of Virginia
JOSEPH, DON R.....	St. Louis University
KAHN, MAX.....	Beth Israel Hospital, N. Y. City
KAHN, MORRIS H.....	Beth Israel Hospital, N. Y. City
KAHN, MORTON C.....	Cornell Medical College, N. Y. City
KAHN, R. L.....	Michigan Department of Health, Lansing
KARSNER, H. T.....	Lakeside Hospital, Cleveland
KAST, LUDWIG.....	N. Y. Post-Graduate Medical School
KELLOGG, V. L.....	National Research Council, Washington, D. C.
KENDALL, ARTHUR I.....	Washington University Medical School, Mo.
KENDALL, E. C.....	Mayo Clinic, Rochester, Minn.
KESSEL, JOHN F.....	Peking Union Medical College, China
KEY, JOHN A.....	Shriner Hospital, St. Louis, Mo.
KILLIAN, J. A.....	N. Y. Post-Graduate Medical School

KINGSBURY, F. B.	Metropolitan Life Insurance Co., N. Y. City
KINSELLA, RALPH A.	St. Louis University
KIRKBRIDE, MARY B.	N. Y. State Dep't of Health, Albany, N. Y.
KIRKHAM, WILLIAM B.	Springfield College, Mass.
KLEINER, I. S.	N. Y. Homeopathic Medical School, N. Y. City
KLIGLER, I. J.	Dep't of Health, Palestine
KLINE, B. S.	Mt. Sinai Hospital, Cleveland, Ohio
KLOTZ, OSKAR	University of Toronto
KNOWLTON, FRANK P.	Syracuse University
KNUDSON, ARTHUR	Albany Medical College
KOBER, PHILIP A.	Kober Chemical Company, Nepera Park, N. Y.
KOCH, MATHILDA L.	Washington, D. C.
KOCHER, R. A.	San Diego, Calif.
KOFOID, CHARLES A.	University of California
KOLMER, JOHN A.	University of Pennsylvania
KOPELOFF, NICHOLAS	Psychiatric Institute, N. Y. City
KORNS, JOHN H.	Peking Union Medical College, China
KRAMER, BENJAMIN	Johns Hopkins University
KRUMBHAAR, E. B.	Philadelphia General Hospital, Philadelphia
KRUMWIEDE, CHARLES	New York University
KRUSE, THEOPHILE K.	University of Pittsburgh
KUGELMASS, I. NEWTON	Yale University
KUNTZ, ALBERT	St. Louis University
LADD, WILLIAM S.	Columbia University
LAMAR, R. V.	University of Georgia
LAMBERT, R. A.	Faculdade de Medicina e Cirurgia, São Paulo, Brazil
LA MER, VICTOR K.	Columbia University
LAMSON, PAUL D.	Johns Hopkins University
LANCEFIELD, D. E.	Columbia University
LANDSTEINER, KARL	Rockefeller Institute, N. Y. City
LANGSTROTH, LOVELL	University of California
LARSON, W. P.	University of Minnesota
LASHLEY, K. S.	University of Minnesota
LATHROP, CARL O.	University of Buffalo
LAUGHLIN, H. H.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
LAURENS, HENRY	Yale University
LEAKE, J. P.	Hygienic Laboratory, Washington, D. C.
LEE, FERDINAND C.	Johns Hopkins University
LEE, FREDERICK S.	Columbia University
LEVENE, P. A.	Rockefeller Institute, N. Y. City
LEVIN, ISAAC	New York City
LEVINE, MICHAEL	Montefiore Hospital, N. Y. City
LEVINSON, SAMUEL A.	University of Illinois
LEVY, ROBERT L.	Presbyterian Hospital, N. Y. City
LEWIS, HOWARD B.	University of Michigan
LEWIS, PAUL A.	Rockefeller Institute, Princeton, N. J.
LEWIS, ROBERT C.	University of Colorado
LIEB, C. C.	Columbia University

LILLIE, FRANK R.....	University of Chicago
LILLIE, RALPH S.....	University of Chicago
LIM, ROBERT K.....	Peking Union Medical College, China
LIPMAN, CHARLES B.....	University of California
LITTLE, C. C.....	University of Maine
LIU, J. HENG.....	Peking Union Medical College, China
LOEB, LEO.....	Washington University
LOEB, ROBERT F.....	Presbyterian Hospital, N. Y. City
LOEVENHART, A. S.....	University of Wisconsin
LOMBARD, WARREN P.....	University of Michigan
LONGCOPE, W. T.....	Johns Hopkins University
LUCAS, WILLIAM P.....	University of California
LUCKE, BALDWIN.....	University of Pennsylvania
LUCKHARDT, A. B.....	University of Chicago
LUND, E. J.....	University of Minnesota
LUNDGAARD, CHRISTEN.....	Medical Clinic A, Copenhagen, Denmark
LUSK, GRAHAM.....	Cornell University Medical College, N. Y. City
LYLE, W. G.....	Roosevelt Hospital, N. Y. City
LYNCH, CLARA J.....	Rockefeller Institute, N. Y. City
LYON, E. P.....	University of Minnesota
MACALLUM, A. B.....	McGill University
MACHT, DAVID I.....	Johns Hopkins University
MACKENZIE, GEORGE M.....	Columbia University
MARRIOTT, MCKIM.....	Washington University
MCCANN, WILLIAM S.....	University of Rochester
MCCLENDON, J. FRANCIS.....	University of Minnesota
MCCCLINTOCK, JOHN T.....	University of Iowa
MCCOLLUM, E. V.....	Johns Hopkins University
MCCRUDDEN, FRANCIS H.....	Boston, Mass.
MCCUTCHEON, MORTON.....	University of Pennsylvania
McELROY, W. S.....	University of Pittsburgh
McJUNKIN, FRANK A.....	Washington University
McLEAN, FRANKLIN C.....	University of Chicago
McMASTER, PHILIP D.....	Rockefeller Institute, N. Y. City
McMEANS, J. W.....	University of Pittsburgh
McQUARRIE, IRVINE.....	Yale University
MACDOUGAL, D. T.....	Desert Laboratory, Tucson, Arizona
MACDOWELL, E. CARLTON..	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
MACLEOD, GRACE.....	Columbia University
MACLEOD, J. J. R.....	University of Toronto
MACNEAL, WARD J.....	N. Y. Post-Graduate Medical School
MACNIDEK, WILLIAM de B.....	University of North Carolina
MAGATH, T. B.....	Mayo Clinic, Rochester, Minn.
MALTANER, FRANK.....	N. Y. State Department of Health, Albany
MANDEL, ARTHUR R.....	New York University
MANDEL, JOHN A.....	New York University
MANN, FRANK C.....	University of Minnesota
MANN, HUBERT.....	United States Veterans' Hospital, N. Y. City

MEMBERS' LIST (ALPHABETIC)

583

MANWARING, W. H.	Leland Stanford University
MARINE, DAVID	Montefiore Hospital, N. Y. City
MARSHALL, E. K. JR.	Johns Hopkins University
MARTIN, E. G.	Leland Stanford University
MATAS, RUDOLPH	New Orleans, La.
MATTILL, HENRY A.	University of Rochester
MAVOR, JAMES A.	Union College
MAXIMOW, ALEXANDER A.	University of Chicago
MAXWELL, S. S.	University of California
MAYNARD, L. A.	Cornell University
MEGRAIL, EMERSON	Western Reserve University
MEHRTENS, HENRY G.	Leland Stanford University
MEIGS, E. B.	Dairy Division Exp. Station, Beltsville, Md.
MELENEY, HENRY E.	Peking Union Medical College, China
MELLON, RALPH R.	Highland Hospital, Rochester, N. Y.
MENDEL, LAFAYETTE B.	Yale University
MENTEN, MAUDE L.	University of Pittsburgh
METZ, CHARLES W.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
MEYER, ADOLF	Johns Hopkins University
MEYER, A. L.	Johns Hopkins University
MEYER, G. M.	Rockefeller Institute, N. Y. City
MEYER, K. F.	University of California
MILLER, C. PHILIP, JR.	Rockefeller Institute, N. Y. City
MILLER, G. H.	University of Iowa
MILLET, JOHN A. P.	Stockbridge, Mass.
MITCHELL, O. W. H.	Syracuse University
MOORE, A. R.	Rutgers College
MORGAN, T. H.	Columbia University
MORSE, ARTHUR	New Haven Hospital
MORSE, WITHROW	Jefferson Medical College
MOSENTHAL, H. O.	N. Y. Post-Graduate Medical School
MUDD, STUART	Rockefeller Institute, N. Y. City
MUDGE, B. S.	University of California
MUELLER, E. F.	Columbia University
MUELLER, J. HOWARD	Harvard University
MULLER, HERMAN J.	University of Texas
MURLIN, JOHN R.	University of Rochester
MURPHY, J. B.	Rockefeller Institute, N. Y. City
MURRAY, HENRY A.	Cambridge University, England
MURRAY, THOMAS J.	Rutgers College
MUSSER, JOHN H.	Tulane University
MYERS, CHESTER N.	Columbia University
MYERS, VICTOR C.	University of Iowa
NELSON, THURLOW C.	Rutgers College
NICHOLS, J. S.	University of Pittsburgh
NILES, WALTER L.	Cornell University Medical College, N. Y. City
NOBLE, W. C. JR.	New York University
NOGUCHI, H.	Rockefeller Institute, N. Y. City

NORRIS, CHARLES.....	Chief Medical Examiner, N. Y. City
NORTHROP, JOHN H.....	Rockefeller Institute, N. Y. City
NOVY, FREDERICK G.....	University of Michigan
OERTEL, HORST.....	McGill University
OLITSKY, PETER K.....	Rockefeller Institute, N. Y. City
OLIVER, JEAN.....	Lane Hospital, San Francisco, Calif.
OPHÜLS, WILLIAM.....	Leland Stanford University
OPIE, EUGENE L.....	Henry Phipps Institute, Philadelphia
OPPENHEIMER, B. S.....	Columbia University
ORNSTEIN, GEORGE G.....	Columbia University
OSBORNE, THOMAS B.....	Agricultural Exp. Station, New Haven, Conn.
OSTERHOUT, W. J. V.....	Harvard University
OTTENBERG, R.....	Mt. Sinai Hospital, N. Y. City
PACKARD, CHARLES.....	Columbia University
PAGE, IRVINE H.....	Cornell University Medical College, N. Y. City
PALMER, LEROY S.....	University of Minnesota
PALMER, W. W.....	Presbyterian Hospital, N. Y. City
PAPANICOLAOU, GEORGE N.....	Cornell University Medical College, N. Y. City
PAPPENHEIMER, A. M.....	Columbia University
PARK, E. A.....	Yale University
PARK, WILLIAM H.....	New York University
PARKER, GEORGE H.....	Harvard University
PARKER, JULIA T.....	Columbia University
PEABODY, FRANCIS W.....	Boston City Hospital
PEARCE, LOUISE.....	Rockefeller Institute, N. Y. City
PEARL, RAYMOND.....	Johns Hopkins University
PEASE, MARSHALL C.....	N. Y. Post-Graduate Medical School
PEIRCE, GEORGE J.....	Leland Stanford University
PELLINI, EMIL J.....	New York University
PEMBERTON, RALPH.....	Presbyterian Hospital, Philadelphia
PENFIELD, WILDER G.....	Columbia University
PEPPER, O. H. FERRY.....	University of Pennsylvania
PERLZWEIG, WILLIAM A.....	Johns Hopkins Hospital
PERMAR, HOWARD H.....	Mercy Hospital, Pittsburgh
PETERS, JOHN P.....	Yale University
PETERSON, W. F.....	University of Illinois
PETTIBONE, C. J. V.....	University of Minnesota
PFÄFF, FRANZ.....	Harvard University
PFEIFFER, J. A. F.....	Johns Hopkins University
PIKE, F. H.....	Columbia University
PLANT, O. H.....	University of Iowa
PLOTZ, HARRY.....	Pasteur Institute, Paris
POHLMAN, AUGUSTUS G.....	St. Louis University
PORTER, WILLIAM T.....	Harvard University
POWERS, GROVER F.....	Yale University
PRATT, JOSEPH H.....	Boston, Mass.
PREWITT, PROVISIO.....	New York University
PRINCE, A. L.....	Hartford Hospital, Conn.

- RAIZISS, GEORGE W...Research Institute of Cutaneous Medicine, Philadelphia
 RAKESTRAW, NORRIS W.....Leland Stanford University
 RASMUSSEN, A. T.....University of Minnesota
 RATNER, BRET.....New York University
 RAVENEL, M. P.....University of Missouri
 RAY, HENRY M.....South Side Hospital, Pittsburgh
 READ, BERNARD E.....Peking Union Medical College, China
 READ, J. MARION.....Leland Stanford University
 REIMAN, STANLEY P.....University of Pennsylvania
 RETTGER, L. F.....Yale University
 REZNIKOFF, PAUL.....Cornell University
 RHODENBURG, GEORGE L.....Columbia University
 RICHARDS, ALFRED N.....University of Pennsylvania
 RICHARDS, HERBERT M.....Columbia University
 RICHARDSON, HENRY B.....Cornell University Medical College, N. Y. City
 RICHEY, DEWAYNE G.....University of Pittsburgh
 RIDDLE, OSCAR.....Station for Exp. Evolution, Cold Spring Harbor, N. Y.
 RINGER, A. I.....Montefiore Hospital, N. Y. City
 RINGER, MICHAEL.....N. Y. City
 ROBERTSON, H. E.....University of Minnesota
 ROBERTSON, OSWALD H.....Peking Union Medical College, China
 ROBERTSON, T. B.....University of Adelaide, South Australia
 ROBINSON, CHARLES S.....Michigan Agricultural Station, Lansing
 ROBINSON, G. CANBY.....Vanderbilt University
 ROCKWOOD, ELBERT W.....University of Iowa
 ROGERS, FRED T.....Baylor University, Texas
 ROGOFF, J. M.....Western Reserve University
 ROMAN, BENJAMIN.....Buffalo General Hospital, N. Y.
 ROSE, ANTON R.....Prudential Life Insurance Co., Newark, N. J.
 ROSE, MARY SWARTZ.....Columbia University
 ROSE, WILLIAM C.....University of Illinois
 ROSENAU, M. J.....Harvard Medical School
 ROSENOW, E. C.....Mayo Foundation, Rochester, Minn.
 ROSS, VICTOR.....Lehn and Fink, Bloomfield, N. J.
 ROTH, GEORGE B.....George Washington University
 ROTHSCHILD, M. A.....Mt. Sinai Hospital, N. Y. City
 ROUS, PEYTON.....Rockefeller Institute, N. Y. City
 RYAN, A. H.....Tuft's Medical College

 SABIN, FLORENCE R.....Johns Hopkins University
 SALANT, WILLIAM.....University of Georgia
 SALVESEN, HARALD A.....Rikshospitalet, Oslo, Norway
 SANFORD, A. H.....Mayo Clinic, Rochester, Minn.
 SANSUM, W. D.....Santa Barbara Cottage Hospital
 SCAMMON, R. E.....University of Minnesota
 SCHICK, BELA.....Mount Sinai Hospital, N. Y. City
 SCHLESINGER, M. J.....Harvard University
 SCHLOSS, OSCAR M.....Cornell University Medical College, N. Y. City
 SCHLUTZ, F. W.....University of Minnesota

SCHMIDT, CARL F.....	University of Pennsylvania
SCHMIDT, CARL L. A.....	University of California
SCHNEIDER, EDWARD C.....	Wesleyan University
SCHNEIDER, J. P.....	University of Minnesota
SCHULTZ, E. W.....	Leland Stanford University
SCHULTZ, W. H.....	University of Maryland
SCHWYZER, FRITZ.....	Kastanienbaum, Switzerland
SCOTT, E. L.....	Columbia University
SCOTT, F. H.....	University of Minnesota
SCOTT, G. G.....	College of the City of New York
SCOTT, R. W.....	City Hospital, Cleveland, Ohio
SENIOR, HAROLD D.....	New York University
SHAFFER, PHILIP A.....	Washington University
SHAKLEE, A. O.....	St. Louis University
SHANNON, W. R.....	University of Minnesota
SHARLIT, HERMAN.....	Roosevelt Hospital, N. Y. City
SHERMAN, H. C.....	Columbia University
SHERMAN, JAMES M.....	Cornell University
SHERWIN, CARL P.....	Fordham University
SHEVKY, ESHREF.....	Leland Stanford University
SHIBLEY, GERALD S.....	Columbia University
SHIPLE, GEORGE J., S. J.....	Woodstock College, Md.
SHIPLEY, PAUL G.....	Johns Hopkins University
SHIVE, J. W.....	N. J. State Agr. Exp. Station
SHOHL, ALFRED T.....	Yale University
SIA, RICHARD H. P.....	Peking Union Medical College, China
SILER, J. F.....	United States Army
SIMPSON, GEORGE ERIC.....	University of Pennsylvania
SIMPSON, SUTHERLAND.....	Cornell University
SITTENFIELD, M. J.....	Columbia University
SMITH, ARTHUR H.....	Yale University
SMITH, FRED M.....	University of Iowa
SMITH, GEORGE H.....	Yale University
SMITH, PHILIP E.....	University of California
SMITH, THEOBALD.....	Rockefeller Institute, Princeton, N. J.
SMYLY, H. JOCELYN.....	Peking Union Medical College, China
SNYDER, FRANKLIN F.....	University of Rochester
SOLLMANN, TORALD.....	Western Reserve University
SOULE, MALCOLM HERMAN.....	University of Michigan
SPAETH, R. A.....	Medical School, Bangkok, Siam
SPEIDEL, C. C.....	University of Virginia
STADIE, WILLIAM C.....	University of Pennsylvania
STAKMAN, E. C.....	University of Minnesota
STARK, MARY B.....	N. Y. Homeopathic Medical College, N. Y. City
STEVENS, FRANKLIN A.....	Presbyterian Hospital, N. Y. City
STEWART, G. N.....	Western Reserve Medical School
STILES, PERCY G.....	Harvard University
STILLMAN, EDGAR G.....	Presbyterian Hospital, N. Y. City
STILLMAN, RALPH G.....	Cornell University

MEMBERS' LIST (ALPHABETIC)

587

- STOCKARD, CHARLES R.....Cornell University Medical College, N. Y. City
 STOOKEY, LYMAN B.....University of Southern California
 STOREY, THOMAS A.....College of the City of New York
 STRONG, RICHARD P.....Harvard University
 STROUSE, SOLOMON.....Northwestern University
 STURTEVANT, A. H.....Columbia University
 SUGUIRA, KANEMATSU.....Memorial Hospital, N. Y. City
 SUMNER, JAMES B.....Cornell University
 SUNDSTROEM, EDWARD S.....University of California
 SWAIN, R. E.....Leland Stanford University
 SWEET, J. EDMUND.....University of Pennsylvania
 SWETT, FRANCIS H.....Johns Hopkins University
 SWIFT, H. F.....Rockefeller Institute, N. Y. City
 SWINGLE, W. W.....Yale University
 SYMMERS, DOUGLAS.....New York University
- TALBOT, FRITZ B.....Harvard University
 TASHIRO, SHIRO.....University of Cincinnati
 TAYLOR, CHARLES V.....University of California
 TEN-BROECK, CARL.....Peking Union Medical College, China
 TERRY, B. T.....Vanderbilt University
 THATCHER, ROBERT W.....N. Y. Agr. Exp. Station, Geneva, N. Y.
 THOMAS, ARTHUR W.....Columbia University
 THOMAS, J. E.....St. Louis University
 THOMAS, KARL.....Physiologisch-Chemisches Institut, Leipzig, Germany
 THOMAS, WALTER S.....Clifton Springs Sanitarium, N. Y.
 THRO, WILLIAM C.....Cornell University Medical College, N. Y. City
 TISDALL, FREDERICK F.....University of Toronto
 TOLSTOI, EDWARD.....Yale University
 TORREY, HARRY B.....University of Oregon
 TOOKEY, JOHN C.....Cornell University Medical College, N. Y. City
 TOWNE, EDWARD B.....Lane Hospital, San Francisco
 TSEN, EDGAR T. H.....Epidemic Prevention Bureau, Peking, China
 TSO, ERNEST.....Peking Union Medical College, China
 TYZZER, E. E.....Harvard University
- UHLENHUTH, EDUARD.....N. Y. City
 UNDERHILL, FRANK P.....Yale University
 VAN SLYKE, DONALD D.....Rockefeller Institute, N. Y. City
 VAUGHAN, T. WAYLAND.....Scripps Institution, California
 VOGEL, KARL M.....Columbia University
 VON MEYSENBURG, LUDO.....Tulane University
- WADSWORTH, AUGUSTUS B.....N. Y. State Department of Health
 WAKSMAN, S. A.....N. J. State Agr. Experiment Station
 WALKER, E. L.....University of California
 WALLACE, GEORGE B.....New York University
 WARDEN, CARL C.....St. Joseph's Hospital, Ann Arbor, Mich.
 WARTHIN, ALFRED S.....University of Michigan

WASTENEYS, H.....	University of Toronto
WATANABE, C. K.....	Watanabe Hospital, Tokyo, Japan
WEBSTER, LESLIE T.....	Rockefeller Institute, N. Y. City
WEISKOTTEN, HERMAN G.....	Syracuse University
WEISS, CHARLES.....	Research Institute of Cutaneous Medicine, Philadelphia
WEISS, HARRY.....	Columbia University
WEISS, SOMA.....	Cornell University Medical College, N. Y. City
WELKER, W. H.....	University of Illinois
WELLER, CARL V.....	University of Michigan
WEST, RANDOLPH.....	Presbyterian Hospital, N. Y. City
WEYMOUTH, FRANK W.....	Leland Stanford University
WHIPPLE, GEORGE H.....	University of Rochester
WHITE, G. BENJAMIN.....	Antitoxin and Vaccine Laboratory, Boston
WHITE, ORLANDO E.....	Brooklyn Botanical Garden, Brooklyn, N. Y.
WIGGERS, CARL J.....	Western Reserve University
WILLAMAN, J. J.....	University of Minnesota
WILLIAMS, ANNA W.....	Department of Health, N. Y. City
WILLIAMS, HORATIO B.....	Columbia University
WILLIAMS, H. U.....	University of Buffalo
WILLIAMS, J. R.....	Highland Hospital, Rochester, N. Y.
WILLIAMS, ROBERT R.....	Western Electric Company
WILSON, D. WRIGHT.....	University of Pennsylvania
WILSON, EDMUND B.....	Columbia University
WINSLOW, C. E. A.....	Yale University
WINTERNITZ, MILTON C.....	Yale University
WISLOCKI, GEORGE B.....	Johns Hopkins University
WOLBACH, S. B.....	Harvard University
WOLF, C. G. L.....	Addenbrooke's Hospital, Cambridge, England
WOLLSTEIN, MARTHA.....	Babies' Hospital, N. Y. City
WOOD, FRANCIS C.....	Columbia University
WOODRUFF, L. L.....	Yale University
WU, HSIEN.....	Peking Union Medical College, China
YATSU, NAOHIDE.....	Zoological Institute, Tokyo, Japan
YERKES, ROBERT M.....	Yale University
YOULAND, WILLIAM E, JR.....	N. Y. Homeopathic Medical College
YOUNG, C. C.....	Department of Health, Mich.
YOUNG, CHARLES W.....	Peking Union Medical College, China
ZINGHER, ABRAHAM.....	Department of Health, N. Y. City
ZINSSER, HANS.....	Harvard University
ZUCKER, THEODORE.....	Columbia University

Total number of members at the close of the academic year, 1924-25: 695.

MEMBERS' LIST (Institutional)

HONORARY

William T. Councilman, Harvard University.
Edward T. Reichert, University of Pennsylvania.
William H. Welch, Johns Hopkins University.

GREATER NEW YORK

College of the City of New York.—W. W. Browne, A. J. Goldforb, G. G. Scott, T. A. Storey.

Columbia University.—C. H. Bailey, L. Bauman, G. N. Calkins, C. B. Coulter, Maynie R. Curtis, G. Draper, W. H. Eddy, G. A. Friedman, F. P. Gay, W. J. Gies, Louise H. Gregory, B. Harrow, H. L. Heft, J. Gardner Hopkins, J. W. Jobling, V. K. La Mer, D. E. Lancefield, F. S. Lee, C. C. Lieb, G. Mac Leod, E. F. Mueller, T. H. Morgan, B. S. Oppenheimer, G. G. Ornstein, C. Packard, A. M. Pappenheimer, Julia T. Parker, W. G. Pennfield, F. H. Pike, H. M. Richards, G. L. Rhodenburg, Mary S. Rose, E. L. Scott, H. C. Sherman, G. S. Shibley, M. J. Sittenfield, A. H. Sturtevant, A. W. Thomas, K. M. Vogel, H. Weiss, H. B. Williams, E. B. Wilson, F. C. Wood, T. F. Zucker.

Cornell University Medical College.—H. J. Bagg, H. Bailey, S. R. Benedict, R. L. Cecil, R. Chambers, W. H. Chambers, J. W. Churchman, A. F. Coca, E. F. DuBois, H. J. Deuel, D. J. Edwards, C. Eggleston, W. J. Elser, J. Ewing, J. A. Hartwell, R. A. Hatcher, M. C. Kahn, G. Lusk, W. L. Niles, I. H. Page, G. N. Papanicolaou, P. Reznikoff, H. B. Richardson, O. M. Schloss, R. G. Stillman, C. R. Stockard, W. C. Thro, E. Tolstoi, J. C. Torrey, S. Weiss.

Fordham University.—J. Byrne, C. P. Sherwin.

Hospitals. Babies.—Martha Woolstein. *Beth Israel.*—M. Kahn, M. H. Kahn. *Brownsville.*—L. Gross. *Joint Diseases.*—H. L. Jaffe. *Lenox Hill.*—A. Bernhard, R. Burton Opitz, A. L. Garbat. *Long Island College.*—E. Goetsch. *Manhattan Eye, Ear and Throat.*—A. A. Eggston. *Memorial.*—K. Sugura. *Montefiore.*—E. J. Baumann, F. H. Frankel, I. Levin, M. Levine, D. Marine, A. I. Ringer. *Mt. Sinai.*—G. Baehr, B. B. Crohn, C. A. Elsberg, A. A. Epstein, R. Ottenberg, M. A. Rothschild, B. Schick. *New York.*—N. B. Foster, *Presbyterian.*—D. W. Atchley, A. R. Dochez, H. R. Geyelin, W. S. Ladd, R. L. Levy, R. F. Loeb, G. M. Mackenzie, W. W. Palmer, F. A. Stevens, E. G. Stillman, R. West. *Roosevelt.*—K. G. Falk, I. Greenwald, W. G. Lyle, H. Sharlit. *St. Luke's.*—R. H. Boots, L. W. Famulener, *U. S. Veterans' No. 81.*—H. Mann.

New York City Departments. Health.—E. J. Banzhaf, Anna W. Williams, A. Zingher. *Chief Medical Examiner.*—C. Norris.

New York Homeopathic Medical College.—I. S. Kleiner, Mary B. Stark, W. E. Youland, Jr.

New York Post-Graduate Medical School.—C. V. Bailey, A. F. Chace, M. Cohen, C. W. Crampton, R. H. Halsey, L. Kast, J. A. Killian, W. J. MacNeal, H. O. Mosenthal, M. C. Pease.

New York University.—W. H. Barber, H. Brooks, W. Coleman, Helen C. Coombs, H. B. Eisberg, A. O. Gettler, A. F. Hess, H. C. Jackson, C. Krumweide, A. R. Mandel, J. A. Mandel, W. C. Noble, W. H. Park, E. J. Pellini, P. V. Prewitt, B. Ratner, H. D. Senior, D. Symmers, G. B. Wallace.

Psychiatric Institute.—N. Kopeloff.

Rockefeller Institute for Medical Research.—O. T. Avery, C. A. L. Binger, J. Bronfenbrenner, W. H. Brown, A. E. Cohn, R. Cole, S. Flexner, F. L. Gates, A. B. Hastings, M. Heidelberger, W. A. Jacobs, K. Landsteiner, P. A. Levene, Clara J. Lynch, P. D. McMaster, G. M. Meyer, C. P. Miller, S. Mudd, J. B. Murphy, H. Noguchi, J. H. Northrup, P. K. Olitsky, Louise Pearce, P. Rous, H. F. Swift, D. D. Van Slyke, L. T. Webster.

Industrial Laboratories (New York City). Metropolitan Life Insurance Company.—F. B. Kingsbury. *Research Laboratory of H. A. Metz.*—H. E. Dubin. *Berg Biological Laboratory.*—W. N. Berg. *Research Laboratory of H. A. Metz (Brooklyn).*—C. W. Hooper, C. N. Myers. *Western Electric Company.*—R. R. Williams.

Brooklyn Botanic Garden.—C. S. Gager, O. E. White

OUTSIDE GREATER NEW YORK

Agricultural Experiment Stations. Connecticut.—T. B. Osborne. *Maryland.*—E. B. Meigs. *New Jersey.*—J. W. Shive, S. A. Waksman. *Michigan.*—C. L. Robinson. *New York (Geneva).*—R. J. Anderson, R. W. Thatcher.

Carnegie Institution of Washington. (Station for Experimental Evolution, Cold Spring Harbor, N. Y.).—A. M. Banta, A. F. Blakeslee, C. B. Davenport, H. H. Laughlin, E. C. MacDowell, C. W. Metz, O. Riddle. *(Desert Laboratory, Tucson, Ariz.).*—D. T. MacDougal.

State Boards of Health. Michigan.—R. L. Kahn, C. C. Young. *New York.*—Mary B. Kirkbride, F. Maltaner, A. B. Wadsworth.

Hospitals. Addenbrooke's (Cambridge, England).—C. G. L. Wolf. *Alleghany County (Pittsburgh, Pa.).*—G. L. Hoffman. *Barnes Hospital (St. Louis, Mo.).*—H. L. Alexander. *Buffalo City.*—B. Roman. *Boston City (Boston, Mass.).*—F. W. Peabody. *City Hospital (Cleveland, Ohio).*—R. W. Scott. *(Spartansburg, S. C.).*—Katherine R. Collins. *Highland (Rochester, N. Y.).*—R. R. Mellon, J. R. Williams. *Hartford Hospital (Hartford, Conn.).*—A. L. Prince. *Hospital for Sick Children (Toronto).*—F. F. Tisdall. *Lane Hospital (San Francisco).*—A. W. Hewlett, J. Oliver. *Mercy (Pittsfield, Mass.).*—T. Flournoy. *Mercy Hospital (Pittsburgh, Pa.).*—H. H. Permer. *Mt. Sinai Hospital (Ohio).*—B. S. Kline. *Peter Bent Brigham (Boston).*—

M. Ringer. *Philadelphia General*.—E. B. Krumbhaar. *Presbyterian (Philadelphia, Pa.)*.—R. Pemberton. *Baerums Sykehus (Sandviken, Norway)*.—H. F. Höst. *Shriner (St. Louis.)*.—J. A. Key. *South Side (Pittsburgh, Pa.)*.—H. M. Ray.

Institutes. Antitoxin and Vaccine Laboratory (Boston).—B. White. *Clifton Springs Sanitarium (Clifton Springs)*.—R. S. Hubbard. W. S. Thomas. *Research Institute of Cutaneous Medicine (Philadelphia)*.—G. W. Raiziss, C. Weiss. *Gratwick Laboratory (Buffalo)*.—C. F. Cori. *Juvenile Psychopathic (Chicago)*.—H. M. Adler. *Lehn Fink Co., N. J.*—V. Ross. *Mulford Co., Pa.*—F. M. Huntoon. *Potter Metabolic Clinic (Santa Barbara, Calif.)*.—N. R. Blatherwick, W. D. Sansum. *Pasteur Institut (Paris, France)*.—H. Plotz. *Phipps (Philadelphia)*.—E. L. Opie. *Rockefeller (Princeton)*.—L. Florence, F. S. Jones, Paul A. Lewis, T. Smith. *Scripps*.—T. W. Vaughn. *State Epedemiological, Warsaw, Poland*.—C. Funk. *Wistar (Philadelphia)*.—H. H. Donaldson, F. S. Hammett.

U. S. Departments. Bureau of Chemistry (Washington, D. C.).—F. A. Csonka. *Dairy Division (Washington, D. C.)*.—G. E. Holm. *Division of Animal Husbandry*.—P. E. Howe, L. M. Koch. *Hygienic Laboratory (Washington, D. C.)*.—Mathilda M. Brooks, S. C. Brooks, B. Cohen, J. P. Leake. *Surgeon General's Office (Washington, D. C.)*.—J. F. Siler.

National Research Council, Washington, D. C.—V. L. Kellogg.

Universities. Adelaide (South Australia).—T. B. Robertson. *Alberta*.—J. B. Collip. *Alabama*.—C. Brooks. *Amherst*.—O. Glaser. *Baylor*.—F. T. Rogers. *Boston*.—S. B. Hooker. *Buffalo*.—W. J. Atwell, F. R. Griffith, Jr., F. A. Hartman, C. O. Lathrop, H. U. Williams. *Cambridge (England)*.—H. A. Murray. *California*.—W. C. Alvarez, T. D. Bechwith, T. C. Burnett, G. W. Clark, M. S. Dunn, F. C. Eberson, H. M. Evans, E. C. Fleischer, G. L. Foster, D. M. Greenberg, S. J. Holmes, S. H. Hurwitz, C. A. Kofoed, L. Langstroth, C. B. Lipman, W. P. Lucas, S. S. Maxwell, K. F. Meyer, B. S. Mudge, C. L. A. Schmidt, P. E. Smith, E. S. Sundstroem, C. V. Taylor, E. L. Walker. *Chicago*.—A. J. Carlson, C. M. Child, I. S. Falk, F. R. Lillie, R. S. Lillie, A. B. Luckhardt, F. C. McLean, A. A. Maximow. *Cincinnati*.—M. H. Fischer, D. E. Jackson, S. Tashiro. *Clark*.—W. H. Cole. *Copenhagen*.—A. Fischer, L. S. Fridericia, C. Lundsgaard. *Colorado*.—R. C. Lewis. *Cornell*.—C. M. Carpenter, P. A. Fish, S. A. Goldberg, W. A. Hagan, I. C. Hall, C. E. Hayden, L. A. Maynard, J. M. Sherman, S. Simpson, J. B. Sumner. *Edinburgh*.—H. W. Davies. *George Washington*.—E. F. Bostrom, G. B. Roth. *Georgia*.—R. V. Lamar, W. Salant. *Harvard*.—W. C. Boeck, W. B. Cannon, J. A. Dawson, S. R. Detwiler, C. A. Doan, J. L. Gamble, W. Hale, L. J. Henderson, R. Hunt, J. H. Mueller, W. J. V. Osterhout, G. H. Parker, F. Pfaff, W. T. Porter, M. J. Rosenau, M. J. Schlesinger, P. G. Stiles, R. P. Strong, F. B. Talbot, E. E. Tyzzer, S. B. Wolbach, H. Zinsser. *Illinois*.—O. Bergeim, S. A. Levinson, W. F. Peterson, W. C. Rose, W. H. Welker. *Iowa*.—Amy L. Daniels, F. H. Falls, O. H. Gaebler, R. B. Gibson, P. C. Jeans, J. T. McClintock, G. H. Miller, V. C. Myers, H. Plant, E. W. Rockwood, F. M. Smith. *Iowa State (Ames)*.—G. G. De Bord. *(Japan)*.—

N. Yatsu. *Jefferson*.—W. Morse *Johns Hopkins*.—J. J. Abel, H. L. Amoss, J. H. Brown, C. C. Bull, E. P. Carter, R. S. Cunningham, G. A. Harrop, E. C. Hill, L. E. Holt, Jr., W. H. Howell, J. Howland, H. S. Jennings, B. Kramer, P. D. Lamson, F. C. Lee, W. T. Longcope, D. I. Macht, E. K. Marshall, Jr., E. V. McCollum, A. Meyer, A. L. Meyer, R. Pearl, W. A. Perlzweig, J. A. F. Pfeiffer, Florence R. Sabin, P. G. Shipley, F. H. Swett, G. B. Wislocki. *Leipzig*.—K. Thomas. *Leland Stanford*.—T. Addis, C. L. Alsberg, G. D. Barnett, J. P. Baumberger, L. B. Becking, J. F. Cowan, F. DeEds, E. C. Dickson, H. K. Faber, P. J. Hanzlik, W. H. Manwaring, E. G. Martin, H. G. Mehrtens, W. Ophüls, G. J. Peirce, N. W. Rakestraw, J. M. Read, E. W. Schultz, E. Shevky, R. E. Swain, E. B. Towne, F. W. Weymouth. *Liverpool*.—J. G. Adami, *Louisville*.—H. G. Barbour. *Maine*.—C. C. Little. *Maryland*.—W. H. Schultz. *McGill (Montreal)*.—H. Oertel, A. B. Macallum. *Michigan*.—C. W. Edmunds, R. A. Gesell, P. Hadley, G. C. Huber, H. B. Lewis, W. P. Lombard, F. G. Novy, M. H. Soule, A. S. Warthin, C. V. Weller. *Minnesota*.—S. Amberg, E. T. Bell, J. L. Bollman, W. M. Boothby, E. D. Brown, H. L. Dunn, C. H. Eckles, G. Fahr, C. P. Fitch, R. A. Gortner, R. G. Green, J. A. Harris, H. R. Helmholtz, P. S. Hench, A. T. Henrici, A. D. Hirschfelder, C. M. Jackson, E. C. Kendall, C. S. Lashley, W. P. Larson, E. J. Lund, E. P. Lyon, T. B. Magarth, F. C. Mann, J. F. McClendon, L. S. Palmer, C. J. V. Pettibone, A. T. Rasmussen, H. E. Robertson, E. C. Rosenow, A. H. Sanford, R. E. Scammon, F. W. Schlutz, J. P. Schneider, F. H. Scott, W. R. Shannon, E. C. Stakman, J. J. Willaman. *Missouri*.—E. Allen, M. P. Ravenel. *Nebraska*.—A. E. Guenther. *North Carolina*.—W. deB. MacNider. *Norway*.—H. A. Salvesen. *Northwestern*.—L. R. Dradstedt, S. Strouse. *Ohio State*.—R. G. Hoskins. *Oregon*.—H. B. Torrey. *Peking Union Medical*.—J. H. Bauer, A. M. Boring, J. R. Cash, K. K. Chen, E. W. H. Cruickshank, E. D. Congdon, F. R. Dieuaide, E. C. Faust, R. Guy, H. J. Howard, J. F. Kessel, J. H. Korn, R. K. Lim, J. H. Liu, H. E. Meleney, B. E. Read, O. H. Robertson, R. H. P. Sia, H. J. Smyly, C. Ten Broeck, E. T. H. Tsen, E. Tso, H. Wu, C. W. Young. *Pennsylvania*.—A. C. Abbott, J. H. Austin, A. K. Balls, H. C. Bazett, D. H. Bergey, S. Goldschmidt, L. Jonas, J. A. Kolmer, B. Lucké, M. McCutcheon, O. H. P. Pepper, S. P. Reimann, A. N. Richards, C. F. Schmidt, G. E. Simpson, W. C. Stadie, J. E. Sweet, D. W. Wilson. *Pennsylvania State*.—R. A. Dutcher. *Pittsburgh*.—C. G. Guthrie, S. R. Haythorn, D. Hooker, T. K. Kruse, M. L. Menten, W. S. McEllroy, J. M. McMeans, J. S. Nicholas, deW. G. Richey. *Princeton*.—E. G. Conklin, E. N. Harvey. *Rochester*.—S. Bayne-Jones, W. R. Bloor, H. Clough, G. W. Corner, A. F. Guttmacher, W. S. McCann, H. A. Mattill, J. R. Murlin, F. F. Snyder, G. H. Whipple. *Rutgers*.—J. F. Anderson, W. J. Crozier, A. R. Moore, T. C. Nelson. *São Paulo (Brazil)*.—R. A. Lambert. *Southern California (Los Angeles)*.—L. B. Stookey. *Springfield (Mass.)*.—W. B. Kirkham. *St. Louis*.—J. Auer, A. P. Briggs, D. H. Dolley, E. A. Doisy, M. S. Fleisher, W. W. Graves, D. R. Joseph, R. A. Kinsella, A. G. Pohlman, A. O. Shaklee, J. E. Thomas. *Siam*.—R. A. Spaeth. *Syracuse*.—R. K. Brewer, M. S. Dooley, L. M. Hickernell, F. P. Knowlton, O. W. H. Mitchell. *Texas*.—C. Hartman, B. M. Hendrix, H. J. Muller. *Tohoku Imperial (Japan)*.—S. Hatai. *Toronto*.—A. H. Caulfield, J. G. Fitzgerald, W. L. Holman, A. Hunter, O.

Klotz, J. J. R. Macleod, H. Wasteneys. *Tufts*.—F. H. McCrudden, A. H. Ryan. *Tulane*.—R. Ashman, C. Bass, M. J. Couret, H. Cummins, W. Denis, C. W. Duval, W. E. Garrey, H. N. Gould, I. Hardesty, W. H. Harris, F. M. Johns, L. Von Meysenbug, J. H. Musser. *Union (Albany Medical College)*.—W. M. Baldwin, M. Dresbach, A. Knudson. *(Schenectady)*.—J. W. Mavor. *Vanderbilt (Nashville)*.—G. E. Cullen, G. C. Robinson, B. T. Terry. *Virginia*.—H. E. Jordan, C. C. Speidel. *Washington (St. Louis)*.—D. P. Barr, M. T. Burrows, J. V. Cooke, J. Erlanger, E. A. Graham, A. I. Kendall, L. Loeb, M. Marriott, F. A. McJunkin, P. A. Shaffer. *Wesleyan*.—E. C. Schneider. *Western Reserve (Cleveland)*.—M. Collett, G. W. Crile, A. B. Eisenbrey, H. T. Karsner, E. Megrail, J. M. Rogoff, T. Sollman, G. N. Stewart, C. J. Wiggers. *West Virginia*.—F. E. Chidester. *Wisconsin*.—C. R. Bardeen, C. H. Bunting, L. J. Cole, P. F. Clark, J. A. E. Eyster, A. S. Loevenhart. *Woodstock (Md.)*.—G. J. Shiple. *Yale*.—G. A. Baitsell, F. G. Blake, H. S. Burr, R. H. Chittenden, G. R. Cowgill, Hilda M. Croll, E. G. Gross, R. G. Harrison, S. C. Harvey, I. N. Kugelmass, H. Laurens, I. McQuarrie, L. B. Mendel, A. H. Morse, E. A. Park, J. P. Peters, G. F. Powers, L. F. Rettger, A. T. Shohl, A. H. Smith, G. H. Smith, W. W. Swingle, F. P. Underhill, C. E. A. Winslow, L. L. Woodruff, M. C. Winternitz, R. M. Yerkes.

Industrial Laboratories. Battle Creek, Mich.—M. S. Fine. Chicago, Ill., Northwestern Yeast Co.—M. H. Givens. Duxbury, Mass., Food Research Laboratory.—P. B. Hawk. Indianapolis, Ind., Eli Lilly and Co.—G. H. A. Clowes. Nepera Park, N. Y., Kober Chemical Co.—P. A. Kober. Newark, N. J., Prudential Life Insurance Co.—A. R. Rose. Philadelphia, Pa., 1524 Chestnut St., H. H. Mulford Co.—T. S. Githens.

Boston, Mass.—J. H. Pratt. Chattanooga, Tenn.—H. C. Embrey. Los Angeles, Calif.—B. M. Allen. Missouri Botanical Garden, St. Louis, Mo.—B. M. Duggar. New Orleans, La.—I. Cohn, J. Halsey, R. Matas. Norway.—H. A. Salvesen. Ossining, N. Y., R. F. D. 2.—H. D. Dakin. Palestine Board of Health.—I. J. Kligler. San Diego, Calif.—R. A. Kocher. Stockbridge, Mass.—J. A. P. Millet. Tuckahoe, N. Y.—I. F. Harris. Berlin, Germany.—Rhoda Erdmann. Berne, Switzerland.—L. Asher. Kastanienbaum, Switzerland.—F. Schwyzer. Tokyo, Japan.—C. K. Watanabe.

OFFICERS 1903—1925

	Pres.	Vice-Pres.	Treas.	Sec'y.	Additional Members of Council*
1903-04.....	Meltzer	Park	Calkins	Gies	
1904-05.....	Meltzer	Ewing	Calkins	Gies	
1905-06.....	Wilson	Dunham	Calkins	Gies	
1906-07.....	Flexner	Dunham	Calkins	Gies	
1907-08.....	Flexner	Morgan	Calkins	Gies	
1908-09.....	Lee	Morgan	Lusk	Gies	
1909-10.....	Lee	Gies	Lusk	Opie	
1910-11.....	Morgan	Gies	Lusk	Opie	
1911-12.....	Morgan	Levene	Lusk	Wallace	
1912-13.....	Ewing	Levene	Norris	Wallace	
1913-14.....	Ewing	Field	Norris	Jackson	
1914-15.....	Lusk	Gies	Murlin	Jackson	Gies, Auer
			Sec'y.-Treas.		
1915-16.....	Lusk	Calkins	Jackson	Auer, DuBois	
1916-17.....	J. Loeb	Gies	Jackson	DuBois, Wallace	
1917-18.....	Gies	Auer	Jackson	Wallace, Sherman	
1918-19.....	Gies	Auer	Jackson	Sherman, Jobling	
1919-20.....	Calkins	Wallace	Jackson	Jobling, Hess	
1920-21.....	Calkins	Wallace	Jackson	Hess, Myers	
1921-22.....	Wallace	Jobling	Jackson	Myers, DuBois	
1922-23.....	Wallace	Jobling	Myers	DuBois, Benedict	
1923-24.....	Jackson	Jobling	Goldforb	Benedict, Rous	
1924-25.....	Jackson	Jobling	Goldforb		
		{ Iowa Branch Minnesota Branch Southern Branch Pacific Coast Branch Peking (China) Branch St. Louis Branch Western New York Branch		John T. McClintock Frederick H. Scott Charles W. Duval William Ophuls J. Heng Liu Leo Loeb Sutherland Simpson	
Vice-Presidents ex officio (Chairmen of Branches)		{ Iowa Branch Minnesota Branch Southern Branch Pacific Coast Branch Peking (China) Branch St. Louis Branch Western New York Branch		G. H. Miller Frederick H. Scott Irving Hardesty Carl L. A. Schmidt John F. Kessel John Auer James B. Sumner	
Secretaries of Branches		{ Iowa Branch Minnesota Branch Southern Branch Pacific Coast Branch Peking (China) Branch St. Louis Branch Western New York Branch			

*The Past Presidents are also members.

AUTHORS' INDEX

(The numeral indicates the page.)

- A** BRAHAMSON, E. M. (and Miller, E. G., Jr) Hydrogen ion concentration in the gastrointestinal tract of the albino rat 438
- ADDIS, T. (and MAC KAY, L. M., and MAC KAY, E. M.) Compensatory hypertrophy of the kidney: The effect of pregnancy and of lactation..... 536
- ALBUS, W. R. See HOLM, GEORGE E.
- ALLEN, EDGAR (and DOISY, EDWARD A.) Continuation of secretion of the ovarian follicular hormone by the human *Corpus luteum* 303
- ALLEN, R. S. See MURLIN, J. R.
- ALLEN, THOMAS DYER. See KOPPANYI, THEODORE.
- ALSBERG, CARL L. (and PERRY, E. E.) The effect of grinding upon starch and starch pastes 60
- ANDRUS, E. COWLES (and DRURY, A. N.) Conduction in the mammalian auricle as affected by changes in hydrogen ion concentration 21
- ASHER, LEON (and TAKAHASHI, KISHI) On the experimental production of lack of carbohydrates, and on the carbohydrate metabolism of the central nervous system..... 238
- ATWELL, WAYNE G. J. Quantitative studies on the *pars tuberalis* of the *hypophysis cerebri* 499
- AUER, JOHN. On the function of the colonic spindle (*Fusus coli*) of the rabbit..... 331
- AUER, JOHN. Further note on the *fusus coli* of the rabbit..... 301
- B**AGG, HALSEY J. The functional activity of the breast in relation to mammary carcinoma in mice 419
- BAGG, HALSEY J. Hereditary visceral abnormalities in the descendants of irradiated mice 271
- BANTA, ARTHUR M. (and BROWN, L. A.) Rate of metabolism and sex determination in Cladocera 77
- BANTA, A. M. (and SATINA S.) Biochemical reaction with sex in *Cladocera*..... 466
- BANZHAF, EDWIN J. The distribution of the immune bodies occurring in types I, II and III antipneumococcus serum.... 329
- BANZHAF, EDWIN J. (and POVITZKY, OLGA R.) Diphtheria toxin-antitoxin titration by Ramon method for practical application 11
- BARBOUR, H. C. (and HAMILTON, W. F.) Epinephrin anhydremia and its relation to the emergency function of the adrenals 480
- BARNETT, G. D. (and LEWIS, J. K., and HEWLETT, A. W.) The effect of training on lactic acid secretion 537
- BAUER, J. H. See TENBROECK, C.
- BAYLEY, E. C. See SCOTT, F. H.
- BAYNE-JONES, STANHOPE. Heat from reactions between antigens and antibodies. Special reference to diphtheria toxin and antitoxin 246
- BAZETT, H. C. (and TYCHOWSKI, W. Z., and CROWELL, C.) Estimation of blood sugar in decerebrate animals 39
- BECKING, L. B. The identity of the pigments in the purple bacteria 523
- BECKING, L. B. The source of energy of the sulphur bacteria 127
- BECKING, L. B. (and GREGERSEN, M. I.) The effect of light on the permeability of lecithin 130
- BECKING, L. B. (and CHAMBERLIN, JOSEPH C.) A note on the refractive index of chitin 256
- BECKING, L. B. See IRVING, L.
- BEERMAN, PHILIP. See KOPPELOFF, NICHOLAS.
- BENEDICT, STANLEY R. The determination of blood-sugar.... 237
- BENEDICT, E. M. See WEST, R.

- BERG, WILLIAM N. Twelve per cent dextrose media for prolonged anerobe growth..... 91
- BERG, WILLIAM N. Diluting lipoid antigen with a constant dropping syphon 146
- BIRKHAUG, KONRAD E. A study of the biology of streptococcus erysipclatis 292
- BLOOR, W. R. (and GILETTE, ETHYLN M.) The utilization of fat in diabetes..... 251
- BODECKER, C. F. See GIES, W. J.
- BOOHER, LELA E. See MYERS, VICTOR C.
- BOWMAN, H. H. M. (and YEE, MARTIN A.) Crystals of Vitamin B from the Mung bean..... 228
- BOYD, J. D. (and HINES, H. M., and LEESE, C. E.) Study of response to continuous intravenous injection of large amounts of glucose 509
- BRAND, ERWIN (and SANDBERG, MARTA.) Note on the relationship between insulin and trypsin 428
- BRIGGS, A. P. See DOISY, EDWARD A.
- BRISTOL, P. See FLEISCHNER, E. C.
- BRONFENBRENNER, J. (and REICHERT, P.) The flocculation of botulinus toxin antitoxin mixtures 391
- BRONFENBRENNER, J. Further studies on so-called bacteriophage 81
- BROOKS, M. M. The effects of varying internal and external pH of Valonia upon penetration of arsenic 148
- BROOKS, S. C. The effect of washing on the resistance of erythrocytes to hypotonic hemolysis 83
- BROWN, L. A. See BANTA, ARTHUR M.
- BUNTING, R. W. (and PALMERLEE, FAITH.) The rôle of *B. acidophilus* in dental caries 296
- BURR, HAROLD S. See HARVEY, SAMUEL C.
- BURROWS, MONTROSE T. Studies to determine the biological significance of the vitamins 241
- CALKINS, L. A. See SCAMMON, R. E.
- CERVENKA, CHARLES. See HIRSCHFELDER, ARTHUR D.
- CHAMBERLIN, JOSEPH C. See BECKING, L. B.
- CHAMBERS, ROBERT (and REZNIKOFF, PAUL.) The effect of immersing and tearing amœbæ in salt solutions..... 386
- CHAMBERS, ROBERT (and REZNIKOFF, PAUL.) The reaction of the protoplasm of the living amœbæ to injected salts 320
- CHAMBERS, WILLIAM H. See DEUEL, HARRY J., JR.
- CHAMBERS, WILLIAM H. (and DEUEL, H. J., JR.) The metabolism of glycerol in phlorhizin diabetes 273
- CHEER, SHEO-NAN. See ROBERTSON, O. H.
- CHEN, K. K. The effect of ephedrine on digestive secretion 570
- CHEN, K. K. The effect of repeated administration of ephedrine 568
- CHEN, K. K. The effect of ephedrine on experimental shock and hemorrhage 203
- CHEN, K. K. The acute toxicity of ephedrine 404
- CHOWN, H. BRUCE. See HOLT, L. EMMETT, JR.
- CHURCHMAN, J. W. Melanuria in mental disease..... 135
- CLARK, ADA R. See GAY, FREDERICK P.
- COHEN, MARTIN. See KILLIAN, JOHN A.
- COHN, ALFRED E. (and CRAWFORD, J. HAMILTON, and ROSENBERGER, H.) Cinematography of skin capillaries in the living human subject..... 89
- COLBY, WOODARD. See LARSON, W. P.
- COLE, WILLIAM H. Does the optic nerve of the frog tadpole regenerate after section.... 476
- COLE, WILLIAM H. (and RICHMOND, EUGENE.) The use of chloretone as an anesthetic for paramecium 231
- COLLENS, WILLIAM S. Perfusion studies on pancreas and liver 367
- COMBIESCU, D. See IONESCU-MIHAESTI, C.
- CONGDON, C. C. See MC CORDOCK, H. A.

- CONGDON, E. D. Incomplete development of *Conchæ* of dogs in nose from which respiratory current was cut off 566
- COOMBS, HELEN C. The action of some derivatives of ergot in peripheral vasomotor exhaustion 327
- COOMBS, HELEN C. The rôle of the dorsal spinal nerve roots in bulbar anemia 328
- COOMBS, HELEN C. The rôle of the accelerator nerves in bulbar anemia 440
- CORI, CARL F. (and CORI, GERTY T.) Comparative study of the sugar concentration in arterial and venous blood during insulin action 72
- CORI, CARL F. A method for the quantitative study of intestinal absorption 495
- CORI, CARL F. The rate of absorption of hexoses and pentoses 497
- CORI, CARL F. (and CORI, GERTY T.) On the carbohydrate metabolism of malignant tumors 254
- CORI, GERTY T. The insulin content of the pancreas and other tissues in animals poisoned with phlorhizin 74
- CORI, GERTY T. See CORI, CARL F.
- COQUELET, OCT. A micro-method for nephelometric estimation of uric acid and purine bases 119
- CRAWFORD, J. HAMILTON. See COHN, ALFRED E.
- CROWELL, C. See BAZETT, H. C.
- CSONKA, FRANK A. (and JONES, D. BREESE.) Proteins of the cotton-seed 226
- CURRAN, HAROLD R. See SHERMAN, JAMES M.
- DANZER, C. S. Observations on the extra-cardiac circulation 217
- DAVIES, H. W. See VAN SLYKE, D. D.
- DAVIS, J. C. See SCOTT, F. H.
- DAWSON, J. A. Inheritance of an abnormality of form in *Paramecium aurelia* 104
- DE BORD, GEORGE G. Reaction to Gram's stain by certain spore-forming bacteria 397
- DE FOREST, DAVID M. See MAVOE, JAMES W.
- DEUEL, H. J., JR. See CHAMBERS, WILLIAM H.
- DEUEL, HARRY J., JR. (and CHAMBERS, WILLIAM H., and EVENGEN, JAMES.) Effect of insulin on the metabolism of dogs under amytal anesthesia 424
- DILL, D. B. See SWAIN, R. E.
- D'IRSA, STEPHEN. The action of strophanthus on the chlorinized heart 530
- DOCHEZ, A. R. (and SHERMAN, LILLIAN.) Some reactions in sensitized guinea pigs to the filtrate of scarlatinal streptococcus 282
- DOISY, EDWARD A. (and BRIGGS, A. P., and WEBER, C. J., and KOECHIG, IRENE.) The formation of lactic acid by depancreatized dogs 57
- DOISY, EDWARD A. See ALLEN, EDGAR.
- DOMM, L. V. Sex-reversal following ovariectomy in the fowl. 28
- DRABKIN, DAVID L. (and SHILKRET, H.) Anhydremia with insulin and water intake 369
- DRESBACH, M. (and WADDELL, K. C.) The emetic action of strophanthidin in cats with denervated hearts..... 371
- DRURY, A. N. See ANDRUS, E. COWLES.
- EBERSON, FREDER'K. Phosphatids of tuberculin with diagnostic and sensitizing properties 346
- EDER, HOWARD. See LARSON, W. P.
- ENRIGHT, J. R. See MANWARING, W. H.
- EPSTEIN, ALBERT A. The action of pepsin on insulin..... 9
- EPSTEIN, ALBERT A. The reactivation of insulin *in vitro* and *in vivo* 422
- EVENGEN, JAMES. See DEUEL, HARRY J., JR.
- FERGUSON, JOHN. See IRVING, LAURENCE.
- FERRIS, HENRY W. See HARVEY, SAMUEL C.
- FISH, PIERRE A. The weight curves of castrated kids..... 248

- FISCHER, N. F. (and LARSON, E.) Experiments with extracts of parathyroid glands 447
- FLEISCHNER, E. C. (and BRISTOL, P.) The Schultz-Dale technique with large guinea pigs and with calcium-free Fleisch-Ringer solution 258
- FLEISHER, MOYER S. Effects of inoculating *monilia* isolated from psoriatic patients into human beings 477
- FLEISHER, MOYER S. (and WILHELMJ, C. M.) Physical-chemical changes of the blood in thyroidectomized guinea pigs 478
- FRANKLIN, ANNA. See HEWLETT, A. W.
- FREEDMAN, L. (and SHERNDAL, A. E.) A new color test for differentiating neorsphenamine from sulfarsphenamine.. 287
- FREEMAN, R. G. See MILLER, E. G.
- FRIES, MARGARET E. Influence of ultra-violet radiation on basal metabolism in children 431
- FRUG, JAMES. See Schmidt, CARL L. A.
- G**AEBLER, O. H. See MURLIN, JOHN R.
- GAEBLER, O. H. (and ROSENE, G. L.) Acid-base balance in pregnancy 513
- GAMBLE, JAMES L. (and MC IVER, MUNROE A.) The factors of dehydration in rabbits following pyloric obstruction 365
- GATES, FREDERICK L. (and GRANT, J. H. B.) Some factors affecting the levels of the serum calcium and phosphorus of normal rabbits 315
- GAY, FREDERICK P. (and ADA R. CLARK.) The reticulo-endothelial system in relation to anti-body formation.... 1
- GESELL, ROB'T (and HERTZMAN, ALBICK B.) Continuous recording changes in hydrogen ion concentration of circulating blood. The relation to respiration 298
- GIES, W. J. Histo-chemical proof of the presence of protein matter in dental enamel.... 175
- GILETTE, ETHYLN M. See BLOOR, W. R.
- GOLDBERG, S. A. See MAYNARD, L. A.
- GOLDSCHMIDT, SAM'L (and LIGHT, ARTHUR B.) The cyanosis of peripheral venous engorgement 87
- GORTNER, R. A. See LEWIS, J. H.
- GRABER, V. C. See SMITH, FRED M.
- GRAHAM, VIOLA A. See SUMNER, JAMES B.
- GRANT, J. H. B. See GATES, FREDERICK L.
- GREEN, R. G. Distemper in the silver fox (*Culpes vulpes*)..... 546
- GREEN, R. G. The mechanism and significance of the fragility test 308
- GREEN, R. G. The fragility of human erythrocytes after treatment with pernicious anemia serums 309
- GREGERSEN, M. I. See BECKING, L. B.
- GUNTHER, L. See LAURENS, H.
- GUNTHER, LEWIS. See LAURENS, HENRY.
- H**ALL, IVAN C. (and HOWITT, BEATRICE.) The anaerobic bacteria of the oral cavity 541
- HALVARSON, H. O. Observations on the measurement of the pH of soap solutions..... 358
- HALVARSON, H. O. The preparation of pure sodium ricinoleate 553
- HALVARSON, H. O. See LARSON, W. P.
- HAMILTON, W. F. See BARBOUR, H. C.
- HANAN, ERNEST B. Experimental hypoglycemia and hyperglycemia in the chick embryo 501
- HANCOCK, E. W. See LARSON, W. P.
- HANSON, ADOLPH M. The hormone of the parathyroid gland 560
- HARKAVY, JOSEPH. A spasm-inciting substance in the sputum during asthmatic attacks.. 225
- HARRIS, J. ARTHUR. The accumulation of chlorides in the leaf tissue fluids of Egyptian cotton with the march of the season 415

- HARRIS, J. ARTHUR (and HOFFMAN, W. F., and LAWRENCE, JOHN V.) Differential absorption of anions by varieties of cotton 350
- HARVEY, SAMUEL C. (and FERRIS, HENRY W.) A physiological study of the development of the collateral circulation in the leg of the dog.... 383
- HARVEY, SAMUEL C. (and BURR, HAROLD S.) An experimental study of the origin of the meninges 52
- HASTINGS, A. B. See VAN SLYKE, D. D.
- HATCHER, R. A. The extraction of alkaloids from blood.... 141
- HATHAWAY, J. C. The inverse relation of iodine and goiter in Utah 183
- HATHAWAY, J. C. Results obtained with the use of the smoke precipitator 559
- HAWLEY, ESTELLE E. The influence of insulin on the respiratory metabolism of normal rabbits 66
- HELMAN, DOROTHY. See HESS, ALFRED F.
- HENRICI, A. T. The rate of spore formation in bacteria.... 197
- HENCH, PHILIP S. The mercury combining power of deproteinized blood 556
- HERTZMAN, ALRICK B. See GESELL, ROBERT.
- HESS, ALFRED F. (and JAFFE, H. L.) The effect of double adrenalectomy on the development of rickets in rats..... 103
- HESS, ALFRED F. (and WEINSTOCK, MILDRED.) Antirachitic properties imparted to lettuce and to growing wheat by ultraviolet irradiation..... 5
- HESS, ALFRED F. (and WEINSTOCK, MILDRED.) Antirachitic properties imparted to inert fluids by ultraviolet irradiation 6
- HESS, ALFRED F. (and WEINSTOCK, MILDRED, and HELMAN, F. D.) The development of antirachitic potency in phytosterol and cholesterol following irradiation 227
- HESS, ALFRED F. (and WEINSTOCK, MILDRED.) Some properties of cholesterol and phytosterol activated by irradiation 319
- HESS, ALFRED F. (and WEINSTOCK, M., and HELMAN, D.) Oil activated by irradiation. II. Separation into an antirachitic and an inactive fraction 76
- HEWLETT, A. W. See BARNETT, G. D.
- HEWLETT, A. W. (and LEWIS, J. K., and FRANKLIN, ANNA.) An experimental study of the effect of stenosis upon the respiratory changes induced by muscular exercise.... 64
- HILL, JUSTINA H. See MACHT, DAVID I.
- HINES, H. M. See BOYD, J. D.
- HINES, H. M. See MC CLINTOCK, J. T.
- HIRSCHFELDER, ARTHUR D. (and CERVENKA, CHAS.) The effect of quinidine on interauricular conduction and irritability in the terrapin's heart 311
- HOFFMAN, W. F. See HARRIS, J. ARTHUR.
- HOFFMAN, W. F. See LEWIS, J. H.
- HOLM, GEORGE E. (and ALBUS, W. R.) The effect of surface tension on the growth of *Lactobacillus vulgaricus* and *Lactobacillus acidophilus* 337
- HOLT, L. EMMETT, JR. (and LA MER, VICTOR K., and CHOWN, H. BRUCE.) The solubility product of tertiary calcium phosphate and its importance in biological systems.. 283
- HONEYWELL, HANNAH E. See RIDDLE, OSCAR.
- HORVATH, A. A. The action of ammonia upon the lungs.... 199
- HOSEPIAN, V. M. See MANWARING, W. H.
- HOWE, PERCY R. See WOLBACH, S. BURT.
- HOWITT, BEATRICE. See HALL, IVAN C.
- HUBBARD, ROGER S. (and WRIGHT, FLOYD R.) An experiment on the effect of sodium bicarbonate and of in-tarvin on the excretion of acetone 70
- HUNTOON, F. M. Pneumococci cultivation in large amounts.... 268

- I**ONESCU-MIHAESTI, C. (and COMBIESCU, D.) Local passive immunity against anthrax infection 110
- INOUE, TAKEO.** Experimental tetany and diet 49
- IRVING, LAURENCE.** Regulation of the hydrogen ion concentration and its relation to metabolism and respiration in the starfish 54
- IRVING, LAURENCE.** The carbonic acid-carbonate equilibrium in sea water 55
- IRVING, LAURENCE** (and BECKING, L. B.) Observations on the metabolism of the corallines 162
- IRVING, LAURENCE** (and FERGUSON, JOHN.) The influence of acidity in the intestine upon the absorption of calcium salts by the blood..... 527
- J**ACKSON, C. M. Spontaneous nephritis and compensatory renal hypertrophy in albino rats on diet deficient in vitamin A.... 410
- JACKSON, HENRY, JR.** The effect of high protein diets on the kidneys of rats 482
- JAFFE, H. L.** See HESS, ALFRED F.
- JOBLING, JAMES W.** A method for obtaining distribution of a therapeutic agent throughout the intestinal tract 487
- JOHNSON, BALBINA.** See SITTENFIELD, M. J.
- JONES, D. BREESE.** See CSONKA, FRANK A.
- K**AHN, M. H. A method for recording continuous blood pressure 166
- KENDALL, EDWARD C.** A quantitative study of the physiologic action of thyroxin 307
- KERPER, ALVER H.** See KUNTZ, ALBERT.
- KESSEL, J. E.** The experimental transfer of certain intestinal protozoa from man to monkeys 206
- KHAW, O. K.** The hatching phenomena of *Clonorchis* ova.... 564
- KILLIAN, JOHN A.** (and COHEN, MARTIN.) The chemical composition of the vitreous humor of animal eyes 445
- KIRKBRIDE, MARY B.** (and WHEELER, MARY W.) Comparison of reactions in individuals to toxins prepared from three strains of scarlet fever streptococci 85
- KIRKBRIDE, MARY B.** (and WHEELER, MARY W.) Reactions induced by intracutaneous injections of toxins of streptococci from scarlet fever 86
- KOECHIG, IRENE.** See DOISY, EDWARD A.
- KOLARS, J. J.** See LEVINE, V. E.
- KOPELOFF, NICHOLAS.** Permanence of results obtained by *L. acidophilus* therapy..... 393
- KOPELOFF, NICHOLAS** (and BEERMAN, PHILIP.) *L. acidophilus* versus *L. bulgaricus* milk feeding 318
- KOPPANYI, THEODORE** (and ALLEN, THOMAS DYER.) Experimental production of intra-ocular hypertension 488
- KOPPANYI, THEODORE** (and ALLEN, THOMAS DYER.) Nervous and pharmacodynamic control of the retinal blood flow 490
- KOPPANYI, THEODORE.** Pseudo-autotomy in albino rat..... 48
- KOPPANYI, THEODORE.** See PEARCY, J. FRANK.
- KRASNOW, F.** See MILLER, E. G., JR.
- KUNTZ, ALBERT** (and KERPER, ALVER H.) The sympathetic innervation of voluntary muscles 23
- KUNTZ, ALBERT** (and KERPER, ALVER H.) Experimental observations on the functional significance of the sympathetic innervation of voluntary muscles 25
- L**A MER, VICTOR K. See HOLT, L. EMMETT, JR.
- LANCEFIELD, R. C.** Antigen relationships of the nucleoproteins from the gram-positive cocci 109
- LANDSTEINER, K.** (and MILLER, C. PHILIP, JR.) On individual differences of the blood of chickens and ducks.... 100
- LANDSTEINER, K.** (and VANDER SCHEER, J.) On the antigens of red blood corpuscles.. 98

- LANDSTEINER, K. (and VAN DER SCHEER, J.) Flocculation reactions with hemolytic immune sera 170
- LANDSTEINER, K. (and VAN DER SCHEER, J., and WITT, DAN H.) Group specific flocculation reactions with alcoholic extracts of human blood..... 289
- LANGLEY, WILSON D. Extractives of muscle: A new iminazol-phosphorus compound 234
- LARSON, E. See FISCHER, N. F.
- LARSON, W. P. (and NELSON, EDMOND.) The antigenic properties of pneumococci and streptococci treated with sodium ricinoleate 357
- LARSON, W. P. (and EVANS, R. D., and NELSON, E.) The effect of sodium ricinoleate upon bacterial toxins, and the value of soap-toxin mixtures as antigens 194
- LARSON, W. P. (and COLBY, WOODARD.) Immunization against scarlet fever using sodium ricinoleate as a detoxifying agent 549
- LARSON, W. P. (and HALVORSON, H. O.) The effect of concentration upon the neutralization of toxin by sodium ricinoleate 550
- LARSON, W. P. (and HANCOCK, E. W., and EDER, HOWARD) Antidiphtheritic immunization using sodium ricinoleate as a detoxifying agent 552
- LASHLEY, K. S. The relation of learning and retention to the extent of cerebral lesions in the rat 413
- LAURENS, H. (and MAYERSON, H. S., and GUNTHER, L.) The effect of light and of darkness on some urinary and blood constituents in the dog.... 171
- LAURENS, HENRY (and MAYERSON, H. S., and GUNTHER, LEWIS.) The effects of radiation on calcium and phosphorus 469
- LAURENS, HENRY (and SOOY, J. W.) The effect of light and of darkness on the growth of the albino rat 112
- LAURENS, HENRY (and SOOY, J. W.) The effect of light and of darkness on blood cell number of the growing albino rat.. 114
- LAURENS, HENRY (and SOOY, J. W.) A method for counting blood platelets in the rat..... 116
- LEVINE, V. E. (and KOLARS, J. J.) The effect of insulin on the morphological blood picture 169
- LAWRENCE, JOHN V. See HARRIS, J. ARTHUR.
- LEE, FERDINAND C. A brief note on the anatomy of the uterine opening of the Fallopian tube 470
- LEE, FERDINAND C. A preliminary note on the physiology of the uterine opening of the Fallopian tube 335
- LEESE, C. E. See BOYD, J. D.
- LEWIS, M. J. See MUELLER, E. F.
- LEWIS, J. H. (and WELLS, H. G., HOFFMAN, W. F., and GORTNER, R. A.) An immunological and clinical study of the alcohol-soluble proteins of cereals 185
- LEWIS, J. K. See BARNETT, G. D.
- LEWIS, J. K. See HEWLETT, A. W.
- LIGHT, ARTHUR B. See GOLDSCHMIDT, SAMUEL.
- LINDSAY, B. (and MEDES, G.) Histological changes in the testis of the guinea pig during scurvy and inanition.... 177
- LITARCZEK, STELLA. See MUELLER, J. HOWARD.
- LOEB, ROBERT F. (and NICHOLS, EMILY G.) The influence of proteins on the diffusibility of calcium 275
- LORD, ELIZABETH M. See MAC DOWELL, E. CARLTON.
- LUCKÉ, BALDWIN. Observations on intravital staining of centrifuged marine eggs..... 305
- LUND, E. J. Preliminary note on the effect of a constant magnetic field on morphogenetic processes 125
- MC CLENDON, G. C. The probable occurrence of xerophthalmia in turkeys 184
- MC CLENDON, J. F. A smoke precipitator used in iodine analysis of food stuffs 558
- MC CORDOCK, H. A. (and CONGDON, C. C.) Suppurative otitis of the albino rat..... 150

- MC CLINTOCK, J. T. (and HINES, H. M.) Physiological action of carnosine 515
- MC CULLOUGH, MARGERY, Quantitative changes in arterial blood-sugar during canine anaphylactic shock 257
- MC CULLOUGH, MARGERY. See MANWARING, W. H.
- MC IVER, MUNROE A. See GAMBLE, JAMES L.
- MC MILLIN, H. C. See WEYMOUTH, F. W.
- MAC DOWELL, E. CARLTON (and LORD, ELIZABETH M.) The sex ratio in litters of mice classified by the total amount of prenatal mortality.. 389
- MAC KAY, E. M. See ADDIS, T.
- MAC KAY, L. M. See ADDIS, T.
- MAC LEOD, F. L. See SHERMAN, H. C.
- MAC DONALD, W. J. Extractives of liver possessing blood pressure reducing properties.... 483
- M**ACHT, DAVID I. Concerning the influence of polarized light on some convulsant drugs 471
- MACHT, DAVID I. The influence of polarized light on the action of some ferments 473
- MACHT, DAVID I. (and HILL, JUSTINA H.) The influence of polarized light on yeast and bacteria 474
- MACKENZIE, GEORGE M. Notes on the mechanism of paroxysmal hemoglobinuria 278
- MANWARING, W. H. (and O'NEILL, F. I., and MC CULLOUGH, MARGERY.) Rôle of the intestinal blood vessels in canine anaphylaxis 398
- MANWARING, W. H. The fundamental physiological mechanism of anaphylaxis 63
- MANWARING, W. H. (and ENRIGHT, J. R., and PORTER, DOROTHY F., and MOY, H. BING.) Further evidence of the rôle of hepatic internal secretions in canine anaphylaxis 61
- MANWARING, W. H. (and HOSEPIAN, V. M., O'NEILL, F. I., and MOY, H. B.) The hepatic anaphylatoxin. Final evidence of its rôle in canine anaphylaxis 123
- MANWARING, W. H. (and O'NEILL, F. I., and MOY, H. B.) Changes in glycogen content of the liver in anaphylaxis 124
- MAVOR, JAMES W. (and DE FOREST, DAVID M.) The relative susceptibility to X-rays of the eggs and sperm of *Arbacia* 19
- MAYERSON, H. S. See LAURENS, HENRY.
- MAYERSON, H. S. See LAURENS, H.
- MAYNARD, L. A. (and GOLDBERG, S. A., and MILLER, R. C.) The influence of sunlight on the mineral nutrition of swine 494
- MEDES, G. See LINDSAY, B.
- MELLON, RALPH R. Observations on an ascospore stage for the parasites of *blascomycosis hominis* 69
- MILLER, C. PHILIP, JR. See LANDSTEINER, K.
- MILLER, E. G., JR. See ABRAHAMSON, E. M.
- MILLER, E. G., JR. (and KRASNOW, F., and FREEMAN, R. G.) Studies on the formation of the streptococcus toxin 467
- MILLER, G. H. See SMITH, FRED M.
- MILLER, R. C. See MAYNARD, L. A.
- MOORE, A. R. Electrical stimulation of luminescence in Ctenophores 80
- MORSE, M. See SCHLUTZ, F. W.
- MOY, H. BING. See MANWARING, W. H.
- MUDD, EMILY B. H. See MUDD, STUART.
- MUDD, STUART (and EMILY B. H. MUDD.) Observations on bacteria in films, and the surface-tension factor in phagocytosis 4
- MUELLER, E. F. See MYERS, C. N.
- MUELLER, ERNST FRIEDRICH (and WIENER, HERBERT J., and WIENER, RENÉE VON E.) On the mechanism of insulin action.... 375

- MUELLER, E. F. (and LEWIS, M. J., and MYERS, C. N.) The relation of adrenalin to the action of insulin upon the blood sugar content 142
- MUELLER, J. HOWARD (and SMITH, DOROTHEA E., and LITARCZEK, STELLA.) Residue antigen from a strain of Friedlander bacillus 373
- MUELLER, J. HOWARD. A chemical study of tuberculin..... 209
- MURLIN, J. R. (and ALLEN, R. S.) Inactivation and reactivation of insulin 492
- MURLIN, JOHN R. (and GAEBLER, O. H.) The influence of insulin in phloridzin diabetes.... 67
- MURLIN, JOHN R. (and PIPER, H. A.) The influence of insulin administered by alimentary tract on the blood sugar of etherized and adrena-
lized animals 68
- MURRAY, L. D. See VAN SLYKE, D. D.
- MYERS, C. N. (and MUELLER, E. F.) Studies on the action of insulin neutralized with alkaline solutions 92
- MYERS, C. N. Biochemical studies on the behavior of the leucocytes after intravenous administration of alkalinized salvarsan 95
- MYERS, C. N. Dyes and penetration of arsenic 441
- MYERS, VICTOR C. (and BOOTHER, LELA E.) The use of the bicolorimeter for the estimation of the hydrogen ion concentration of urine 511
- MYERS, VICTOR C. (and BOOTHER, LELA E.) Observations on the excretion of an acid urine in alkalosis 512
- NELSON, EDMOND. See LARSON, W. P.
- NICHOLS, EMILY G. See LOEB, ROBERT F.
- NISHIMURA, KIKUGORO. The lactic acid content of blood and spinal fluid 322
- NOVELLO, N. JEAN. See SHERWIN, CARL P.
- OLITSKY, PETER K. The action of glycerol on the virus of experimental typhus fever and on *Proteus bacilli* 399
- O'NEILL, F. I. See MANWARING, W. H.
- OTTENBERG, R. (and STENBUCK, F. A.) Studies on purification of antibodies, III 211
- OTTENBERG, R. (and STENBUCK, F. A.) Studies on purification of antibodies, IV 215
- PAGE, IRVINE H. Observations on the theory of tetany 117
- PAGE, IRVINE H. Some effects of phosphates parenterally administered 294
- PALMERLEE, FAITH. See BUNTING, R. W.
- PAPANICOLAOU, GEORGE N. Diagnosis of early human pregnancy by the vaginal smear method 436
- PAPANICOLAOU, GEORGE N. The production of certain distinct types of reactions by the use of ovarian extracts..... 106
- PARKER, JULIA T. The production of an exotoxin by certain strains of *staphylococcus aureus* 14
- PEARCE, LOUISE (and VAN ALLEN, C. M.) Effects of controlled conditions of illumination upon malignancy of transplantable neoplasm 448
- PEARCY, J. FRANK (and KOPPANYI, THEODORE.) A further note on regeneration of the cut spinal cord in fish 17
- PERRY, E. E. See ALSBERG, CARL L.
- PIPER, H. A. See MURLIN, JOHN R.
- PORTER, DOROTHY F. See MANWARING, W. H.
- POVITSKY, OLGA. The Ramon flocculation test for determining potency of scarlatinal antitoxin 426
- POVITSKY, OLGA R. See BANZHAF, EDWIN J.
- REED, B. P. See REED, C. I.
- REED, C. I. (and REED, B. P.) Reflex association of feeding and defecation in young birds (*Troglodytes Aedon*) 295
- REICHERT, P. See BRONFENBRENNER, J.
- REZNIKOFF, PAUL. See CHAMBERS, ROBERT.

- RICH, WILLIS H. See WEY-MOUTH, F. W.
- RICHMOND, EUGENE. See COLE, WILLIAM H.
- RIDDLE, OSCAR (and HONEYWELL, HANNAH E.) Blood calcium in relation to sex in pigeons 222
- RIGGS, LLOYD K. The physiologic properties of some unsaturated hydrocarbons 269
- ROBERTSON, O. H. (and SIA, RICHARD H. P., and WOO, SHU-TAI, and CHEER, SHEONAN.) The occurrence of anti-pneumococcus substances in the blood serum in lobar pneumonia 406
- ROGOFF, J. N. See STEWART, G. N.
- ROSE, ANTON R. (and SCHATTNER, FRED) Preventing glucolysis in blood samples 363
- ROSENBERGER, H. See COHN, ALFRED E.
- ROSENE, G. L. See GAEBLER, O. H.
- ROSENOW, E. C. A precipitin reaction in epidemic poliomyelitis 155
- ROSENOW, E. C. Further studies on the etiology of epidemic hiccough 187
- ROSENOW, E. C. A precipitating and neutralizing antistreptococcus (*scarlatinæ*) horse serum 189
- SANDBERG, MARTHA. See BRAND, ERWIN.
- SCAMMON, R. E. (and CALKINS, L. A.) The relation between body-weight and age of the human fetus 157
- SCAMMON, RICHARD E. (and CALKINS, LEROY A.) Empirical formulæ for the proportionate growth of the human fetus 353
- SCHATTNER, FRED. See ROSE, ANTON R.
- SCHLUTZ, F. W. (and MORSE, M.) A note on the productivity of cod liver oil 555
- SCHMIDT, CARL L. A. (and FRUG, JAMES.) Immunological studies on certain albuminoids 345
- SCOTT, F. H. (and BAYLEY, E. C., DAVIS, J. C., and WHITMAN, W.) The effect of pituitrin on blood and on lymph and urine production 312
- SCHOENHOLZ, P. The production of a hemolytic substance in young liquid cultures of *Cl. botulinum* 121
- SCHULTZ, E. W. Platelet deficiency a factor in diminished coagulability of the blood in anaphylaxis 343
- SHEN, T. C. Chemical studies on the urine of eunuchs 408
- SHERMAN, H. C. (and MACLEOD, F. L.) Relation of vitamin A to growth, reproduction and longevity 75
- SHERMAN, JAMES M. (and CURRAN, HAROLD R.) The germicidal action of milk 15
- SHERMAN, LILLIAN. See DOCHÉZ, A. R.
- SHERNDAL, A. E. See FREEDMAN, L.
- SHERWIN, CARL P. (and NOVELLO, N. JEAN.) Metabolism of some heterocyclic compounds 394
- SHERWIN, C. P. Acetylation as a physiologic reaction 182
- SHIBLEY, GERALD S. The importance of changes in electrical charge in specific bacterial agglutination 276
- SHILKRET, H. See DRABKIN, DAVID L.
- SCHWARTZMAN, GREGORY. The influence of partial inactivation upon the potency of the bacteriophage 324
- SCHWARTZMAN, GREGORY. Observations on lytic principle of weak potency 433
- SCHWARTZMAN, GREGORY. Food accessory substances in bacterial growth 7, 42, 44
- SCHWARTZMAN, GREGORY. The mechanism of shortening of the lag period in bacterial cultures containing certain food accessory substances 178
- SIA, RICHARD H. P. The specific effect of pneumococcus soluble substances on the growth of pneumococci in normal serum-leucocyte mixtures 262
- SIA, RICHARD H. P. See ROBERTSON, O. H.
- SITTENFIELD, M. J. (and JOHNSON, BALBINA.) Ef-

- fect of radiumized media upon tissue cultures *in vitro*..... 464
- SMITH, DOROTHEA E. See MUELLER, J. HOWARD.
- SMITH, FRED M. (and MILLER, G. H., and GRABER, V. C.) The action of adrenalin, pituitrin and acetyl-cholin on the coronary arteries of the rabbit 507
- SUMNER, JAMES B. (and GRAHAM, VIOLA A.) The nature of insoluble urease..... 504
- SMYLY, H. J. Experiments on the administration of tartar emetic by various routes 201
- SOOY, J. W. See LAURENS, HENRY.
- STENBUCK, F. A. See OTTENBERG, R.
- STEWART, G. N. (and ROGOFF, J. M.) Studies on adrenal insufficiency 394
- SVENSSON, RUTH M. A morphological distinction between infective larvæ of *Ancylostoma* and *Necator* 261
- SWAIN, R. E. (and DILL, D. B.) Some biochemical notes on *Ariolimax californicus*, Cooper 118
- SWANSON, W. W. A study of the occurrence of peptide nitrogen in the blood 193
- T**AINTER, MAURICE LANE, Inhibition of the Edema under paraphenylenediamine and relationship of the adrenals 544
- TAKAHASHI, KISHI. See ASHER, LEON.
- TAYLOR, C. V. Cataphoresis of ultramicroscopic particles in protoplasm 533
- TAYLOR, T. C. (and BRAUN, C. E., and SCOTT, E. L.) Ultrafiltration and electro dialysis of insulin 453
- TEN BROECK, C. (and BAUER, J. H.) Studies in immunity to tetanus bacilli 562
- THIENES, CLINTON H. Effects of cholesterol on smooth muscle of intestine and uterus 539
- TORREY, J. C. The isolation of the *B. histolyticus* from the ileocaecal region of two human intestines 137
- TSO, ERNEST. The effect of chemical preservation of eggs upon the stability of their vitamin contents 263
- TSO, ERNEST. A method for the preparation of basal dietary free from Vitamin A..... 265
- TYCHOWSKI, W. Z. See BAZETT, H. C.
- U**NGER, W. BYERS. The relation of contractile and food vacuoles to rhythms in *Paramecium* 333
- V**AN ALLEN, C. M. See PEARCE, LOUISE.
- VAN DER SCHEER, J. See LANDSTEINER, K.
- VAN SLYKE, DONALD D. Gasometric determination of urea with urease 486
- VAN SLYKE, D. D. (and HASTINGS, A. B., MURRAY, L. D., and DAVIES, H. W.) Blood reaction and respiration 82
- W**ADDELL, K. C. See DRESBACH, M.
- WEBER, C. J. See DOISY, EDWARD A.
- WEBSTER, L. T. Changes in virulence and growth characteristics of *bacterium lepi-septicum* following alterations in oxygen tension 139
- WELLS, H. G. See LEWIS, J. H.
- WEINSTOCK, MILDRED. See HESS, ALFRED F.
- WELLS, HERBERT S. The absorption and excretion of carbon-tetrachloride in animals and in man 235
- WEST, R. (and BENEDICT, E. M.) Ketogenesis following the feeding of α -oxystearic ethyl ester 280
- WEYMOUTH, F. W. (and MC MILLIN, H. C., and RICH, WILLIS H.) The regression of age with size, a neglected aspect of growth 339
- WHEELER, MARY W. See KIRKBRIDE, MARY B.
- WHITMAN, W. See SCOTT, F. H.
- WIBLE, CHARLES L. The locus of the action of veratrin on the sciatic nerve of the frog.... 336
- WIENER, HERBERT J. See MUELLER, ERNST FRIEDRICH.
- WIENER, RENEE VON E. See MUELLER, ERNST FRIEDRICH.

- WIERZUCHOWSKI, M. Disappearance of ketone bodies in presence of unoxdized sugar in phlorizinized dogs 425
- WIGHT, TOYNBEE. Notes on *Councilmania lafleuri*..... 517
- WILHELMJ, C. M. See FLEISHER, MOYER S.
- WITT, DAN H. See LANDSTEINER, K.
- WOLBACH, S. BURT (and HOWE, PERCY R.) The effect of the scorbutic state upon the production and maintenance of inter-cellular substances 400
- WOLBACH, S. BURT (and HOWE, PERCY R.) The epithelial tissues in experimental Xerophthalmia 402
- WOO, SHU-TAI. See ROBERTSON, H. P.
- WRIGHT, FLOYD R. See HUBBARD, ROGER S.
- YEE, MARTIN A. See BOWMAN, H. H. M.
- ZINGHER, ABRAHAM. The Ramon flocculation test in relation to the antigenic value of diphtheria toxoid (anatoxin) 454
- ZINGHER, ABRAHAM. Early and late immunity results with scarlatinal streptococcus toxin 460
- ZINGHER, ABRAHAM. Immunity results with diphtheria toxoid and 1/10 L+ mixtures of toxin antitoxin 462
- ZINSSER, HANS. Bacterial allergy and tissue reactions 35

SUBJECT INDEX

(The numeral indicates the page.)

- | | | | |
|---|-----|---|-----|
| A BSORPTION, of calcium salts, influence of acidity in intestine | 527 | ANAPHYLAXIS, fundamental, physiological mechanism | 63 |
| ABSORPTION of carbon-tetrachloride | 235 | ANAPHYLAXIS, platelet deficiency and coagulability | 343 |
| ABSORPTION of hexoses and pentoses, rate | 497 | ANCYLOSTOMA and Necator, distinction between larvæ | 261 |
| ABSORPTION, intestinal, quantitative study | 495 | ANEMIA, bulbar, in spinal nerve roots | 328 |
| ACETONE, effect of sodium bicarbonate and intarvin on the excretion | 70 | ANEMIA, bulbar, rôle of accelerator nerves | 440 |
| ACETYL-CHOLIN, action on coronary arteries | 507 | ANEMIA serums, fragility test of erythrocytes | 309 |
| ACETYLATION as physiologic reaction | 182 | ANEROBE growth, dextrose media | 91 |
| ADRENALS, relationship to edema | 544 | ANESTHETIC, chloretone for paramecium | 231 |
| ADRENAL insufficiency | 394 | ANHYDREMIA, epinephrin, emergency function of adrenals | 480 |
| ADRENALIN, action on coronary arteries | 507 | ANHYDREMIA with insulin and water intake | 369 |
| ADRENALIN, relation to action of insulin upon blood-sugar content | 142 | ANION absorption by varieties of cotton | 350 |
| ADRENALIZED animals, influence of insulin on blood sugar | 68 | ANTHRAX infection, local passive immunity | 110 |
| ADRENALECTOMY, double, effect on development of rickets in rats | 103 | ANTIBODIES, purification, 211, | 215 |
| AGE, regression with size | 339 | ANTIBODY formation, reticulo-endothelial system | 1 |
| AGGLUTINATION, bacterial changes in electrical charge.... | 276 | ANTIGEN (residue) from Friedlander bacillus | 373 |
| ALBUMINOIDS, immunological studies | 345 | ANTIGEN of red blood corpuscles | 98 |
| ALKALOIDS, extraction from blood | 141 | ANTIGENIC properties of pneumococci and streptococci, with sodium ricinoleate | 357 |
| ALKALOSIS, excretion of acid urine | 512 | ANTIGENIC relationships of nucleo-proteins from gram-positive cocci | 109 |
| AMMONIA, action upon lungs.... | 199 | ANTIGENS and antibodies, heat from reactions | 246 |
| AMCEBÆ, immersing and tearing in salt solutions | 386 | ANTIGENIC value of diphtheria toxoid, Ramon flocculation test | 454 |
| ANAEROBIC bacteria of oral cavity | 541 | ANTIGENS, value of soap-toxin mixtures | 194 |
| ANESTHESIA, amytal, effect of insulin on metabolism | 424 | ANTIPNEUMOCOCCUS extracts, removal of extraneous material | 215 |
| ANAPHYLACTIC shock, arterial blood sugar | 257 | ANTIPNEUMOCOCCUS serum, immune bodies | 329 |
| ANAPHYLATOXIN, hepatic..... | 123 | ANTIPNEUMOCOCCUS substances in blood serum in lobar pneumonia | 406 |
| ANAPHYLAXIS, canine, in intestinal blood vessels | 398 | ANTIRACHITIC potency following irradiation | 227 |
| ANAPHYLAXIS, glycogen content of liver | 124 | | |

ANTIRACHITIC properties by ultraviolet irradiation, 5; to lettuce and growing wheat.....	6	BLOOD calcium of normal rabbits	315
ANTISTREPTOCOCCUS (<i>scarlatinæ</i>) horse serum	189	BLOOD cell number of growing albino rat, effect of light.....	114
ANTITOXIN, scarlatinal, Ramon flocculation test	426	BLOOD, deproteinized by mercury	556
ARBACIA, eggs and sperm, relative susceptibility to X-rays.....	19	BLOOD, extraction of alkaloids..	141
ARSENIC, effect of dyes on central nervous system and spinal fluid	441	BLOOD flow, retinal, nervous and pharmacodynamic control..	490
ARSENIC, effects of pH upon penetration	148	BLOOD, lactic acid content	322
ASCOSPORE stage, parasites of <i>blastomycosis hominis</i>	69	BLOOD, influence of acidity in intestine upon absorption of calcium salts	527
ASTHMA, spasm-inciting substance	225	BLOOD, physical-chemical changes after thyroidectomy.....	478
AURICLE, conduction changed by hydrogen ion concentration	2535	BLOOD picture, morphological, effect of insulin	169
AUTOTOMY, pseudo, in albino rat	48	BLOOD platelet deficiency in coagulability of blood in anaphylaxis	343
B ACTERIA in films, and the surface tension factor in phagocytosis, 4; influence of initial hydrogen ion concentration of media on growth promoting effect of tomato extract	7	BLOOD platelets in the rat, counting	116
<i>B. ACIDOPHILUS</i> in dental caries	296	BLOOD pressure, method for recording continuous	166
<i>B. HISTOLYTICUS</i> , isolation from ileo-caecal region	137	BLOOD, preventing glucolysis in samples	363
<i>B. LEPISEPTICUM</i> , virulence and growth changes following alterations in oxygen tension....	139	BLOOD reaction and respiration	82
<i>B. PROTEUS</i> , action of glycerol	399	BLOOD sugar, arterial, during anaphylactic shock	257
BACTERIA, purple, identity of pigments	523	BLOOD sugar, determination of	237
BACTERIA, rate of spore formation	197	BLOOD sugar in decerebrate animals	39
BACTERIA, sulphur, source of energy of	127	BLOOD sugar concentration during insulin action	72
BACTERIAL allergy and tissue reactions	35	BLOOD sugar, influence of insulin on, of etherized and adrenaized animals	68
BACTERIAL cultures, shortening of lag period	178	BLOOD sugar content, relation of adrenalin to action of insulin	142
BACTERIAL growth, hydrogen ion concentration of media, 7, 42, 44		BLOOD sugar, unoxylized, in phlorizinized dogs, disappearance of ketone bodies	425
BACTERIOPHAGE, further studies on so-called	81	BLOOD and urinary constituents in dog, effect of light	171
BACTERIOPHAGE, influence of partial inactivation	324	BLOOD, occurrence of peptide nitrogen	193
BIOCHEMISTRY of <i>Ariolimaz californicus</i> , Cooper	118	BODY-WEIGHT and age of human fetus	157
BLASTOMYCOSIS <i>hominis</i> , ascospore stage	69	BOTULINUS toxin antitoxin mixtures, flocculation	391
BLOOD of chickens and ducks....	100	C ALCIUM salts, influence of acidity in intestine on absorption by the blood	527
BLOOD calcium and sex in pigeons	222	CAPILLARIES, skin, cinematography of in the living, human subject	89
		CARBOHYDRATE deficiency, experimental production	238

- CARBOHYDRATE metabolism
of central nervous system 238
- CARBOHYDRATE metabolism
of tumors 254
- CARCINOMA in mice, func-
tional activity of the breast..... 419
- CARIES, *B. acidophilus* 296
- CARNOSINE, physiological ac-
tion 515
- CASTRATION, weight curves
following 248
- CATAPHORESIS of particles.... 533
- CEREBRAL lesions in rat re-
lated to learning and retention 413
- CHITIN, refractive index..... 256
- CHLORETONE as anesthetic for
paramecium 231
- CHOLESTEROL activated by
irradiation 319
- CHOLESTEROL, antirachitic
potency 227
- CHOLESTEROL, effects on
smooth muscle of intestine and
uterus 539
- CIRCULATION, collateral, phys-
iological study of development 383
- CIRCULATION, extra-cardiac... 217
- COAGULABILITY of blood in
anaphylaxis 343
- COD liver oil, photoactivity..... 555
- CONCHÆ, incomplete develop-
ment in dogs in nose from
which respiratory current was
cut off 566
- CONDUCTION in mammalian
auricle as affected by changes
in hydrogen ion concentration 21
- CORALLINES, metabolism 162
- Corpus luteum*, human, secretion
of ovarian follicular hormone.. 303
- COTTON-SEED, proteins 226
- COUNCILMANIA *lafleurii* 517
- CYANOSIS of peripheral venous
engorgement 87
- D**EHYDRATION following py-
loric obstruction 365
- DENTAL enamel, protein in 175
- DEPANCREATIZED dogs, for-
mation of lactic acid 57
- DEVELOPMENT, incomplete, of
Conchæ of dogs in nose from
which respiratory current was
cut off 566
- DIABETES, phlorhidzin, in-
fluence of insulin 67
- DIABETES, phlorhizin, metab-
olism of glycerol 273
- DIETARY (basal) free from
Vitamin A 265
- DIPHThERIA toxin-antitoxin
titration for practical appli-
cation 11
- DIABETES, use of fat 251
- DIFFUSIBILITY of calcium,
influence of proteins 275
- DIPHThERIA immunization
with sodium ricinoleate 552
- DIPHThERIA toxoid, immunity
results 462
- DIPHThERIA toxoid, Ramon
flocculation test in relation to
antigenic value 454
- DISTEMPER in silver fox..... 546
- DYES, effect on concentration of
arsenic into central nervous
system and the spinal fluid..... 441
- E**DEMA, inhibition under para-
phenylenediamine 544
- ELECTRICAL charge in bacter-
ial agglutination 276
- EMETIC, tartar, administration 201
- EPHEDRINE, acute toxicity..... 404
- EPHEDRINE, on shock and
hemorrhage 203
- EPHEDRINE, effect on digest-
ive secretion 570
- EPHEDRINE, effect of repeated
administration 568
- ERGOT, derivatives in peripheral
vaso-motor exhaustion 327
- ERYTHROCYTES, resistance to
hypnotic hemolysis after wash-
ing 83
- ETHERIZED animals, influence
of insulin on blood sugar 68
- EXCRETION of carbon-tetra-
chloride 235
- EXOTOXIN, production of by
certain strains of staphylococ-
cus aureus 14
- F**ALLOPIAN tube opening,
uterine, anatomy 470
- FALLOPIAN tube, physiology
of uterine opening 335
- FAT in diabetes 251
- FETUS, human, relation between
body-weight and age 157
- FILMS, observations on bacteria,
and the surface tension factor
in phagocytosis 4
- FLEISCH-RINGER solution—
Ca, Schultze-Dale technique 258
- FLOCCULATION reactions with
hemolytic immune sera 170
- FLOCCULATION reactions, spe-
cific, with alcoholic extracts of
of human blood 289
- FRAGILITY test, mechanism
and significance 308

FRAGILITY test of human erythrocytes after anemia serum.....	309	of Valonia, effects upon penetration of arsenic	148
<i>FUSUS coli</i> of rabbit	301, 331	HYDROGEN ion of soap solutions	358
G LYCOLYSIS in blood samples, preventing	363	HYDROGEN ion, regulation relation to metabolism and respiration in the starfish	54
GLUCOSE injection, intravenous	509	HYDROGEN ion concentration of circulating blood, continuous recording changes	298
GLYCEROL, action on virus of experimental typhus fever and on <i>Proteus bacilli</i>	399	HYPERGLYCEMIA in chick embryo	501
GLYCEROL in phlorhizin diabetes	273	HYPERTENSION, intra-ocular, experimental	488
GLYCOGEN content of liver, in anaphylaxis	124	HYPOGLYCEMIA in chick embryo	501
GOITER, relation of iodine	183	<i>HYPOPHYSIS cerebri, pars tuberalis</i>	499
GRAM'S Stain, reaction by certain spore-forming bacteria	397	I MINAZOL-PHOSPHORUS compound: extractives of muscle	234
GROWTH of albino rat, effect of light	112	IMMUNE bodies in anti-pneumococcus serum	329
GROWTH of human fetus	353	IMMUNITY, local passive, against anthrax infection	110
GROWTH, regression of age with size	339	IMMUNITY results with diphtheria toxoid	462
H ATCHING phenomena of <i>Clonorchis ova</i>	564	IMMUNITY results with scarlatinal streptococcus toxin	460
HEAT from reactions between diphtheria toxin and antitoxin	246	IMMUNIZATION, antidiphtheritic with sodium ricinoleate	552
HEART, mammalian, conduction by changes of hydrogen ion	21	IMMUNIZATION against scarlet fever with sodium ricinoleate	549
HEMOLYSIS, hypotonic, resistance of erythrocytes after washing	83	IMMUNOLOGICAL study of alcohol-soluble proteins of cereals	185
HEMOGLOBINURIA, paroxysmal	278	IMMUNOLOGICAL studies on albuminoids	345
HEMOLYTIC production in young liquid cultures of <i>Cl. botulinum</i>	121	INANITION, changes in testis of guinea pig	177
HEMORRHAGE, effect of epheдрine	203	INERT fluids, antirachitic properties by ultraviolet irradiation	5
HEPATIC anaphylatoxin, rôle in canine anaphylaxis	123	INHERITANCE of abnormality in <i>Paramecium aurelia</i>	104
HEREDITARY visceral abnormalities in irradiated mice	271	INNERVATION, sympathetic, of voluntary muscles	23
HICCOUGH, epidemic, etiology..	187	Experimental observations on functional significance	25
HORMONE, ovarian follicular, secretion by human <i>corpus luteum</i>	303	INSULIN action, mechanism	375
HYDROCARBONS, unsaturated, physiologic properties	269	INSULIN, action of pepsin on	9
HYDROGEN ion concentration in gastro-intestinal tract of albino rat	438	INSULIN action, blood sugar concentration	72
HYDROGEN ion concentration of media, influence of initial, on growth promoting effect of tomato extract on bacteria 7, 42, 44	21	INSULIN and trypsin	428
Conduction in mammalian auricle affected by	21	INSULIN, anhydremia	369
HYDROGEN ion concentration of urine, use of bicolorimeter..	511	INSULIN content of pancrease in animals poisoned with phlorhizin	74
HYDROGEN ion concentration		INSULIN ultrafiltration	453

- INSULIN, effect on metabolism of dogs under amytal anaesthesia 424
 INSULIN, effect upon morphological blood picture 169
 INSULIN, inactivation 492
 INSULIN, influence on blood sugar of etherized and adrena-
 lized animals 68
 INSULIN, influence in phlorid-
 zin diabetes 67
 INSULIN, influence on respira-
 tory metabolism of rabbits 66
 INSULIN neutralized with alka-
 line solutions 92
 INSULIN, relation of adrenalin
 to action of insulin upon blood-
 sugar content 142
 INSULIN reactivation 492
 INSULIN, reactivation *in vivo*
 and *in vitro* 422
 INTARVIN, effect on the excre-
 tion of acetone 70
IN VITRO, effect of radiumized
 media upon tissue cultures 464
 IODIN analysis of food stuffs by
 smoke 558
 IODIN precipitation by smoke 559
 IODIN, relation to goiter 183
 IRRADIATION, antirachitic po-
 tency 227
 IRRADIATION, cholesterol and
 phytosterol activated 319
 IRRADIATION, oil activated 76
 IRRADIATION of mice, hered-
 itary visceral abnormalities 271

KETOGENESIS following feed-
 ing of α -oxystearic ethyl ester 280
 KETONE bodies, disappearance
 in presence of unoxdized sug-
 ar in phlorizinized dogs 425
 KIDNEY, hypertrophy with
 pregnancy 536

LACTIC acid content of blood
 and spinal fluid 322
 LACTIC acid excretion after
 training 537
 LACTIC acid excretion after
 oxygenated breathing 538
 LACTIC acid, formation by de-
 pancreatized dogs 57
L. acidophilus, effect of surface
 tension 337
L. acidophilus therapy 393
L. acidophilus versus *L. Bulgar-*
icus milk feeding 318
L. Bulgaricus, effect of surface
 tension 337

L. Bulgaricus versus *L. acidoph-*
ilus milk feeding 318
 LAG period in bacterial cultures 178
 LECITHIN, effect of light on
 permeability 130
 LEUCOCYTES, behavior after
 intravenous administration of
 alkalinized salvarsan 95
 LIGHT and darkness, on urinary
 and blood constituents in dog.. 171
 LIGHT, effect on blood cell num-
 ber of growing albino rat 114
 LIGHT, effect on growth of albi-
 no rat 112
 LIGHT, effect on permeability of
 lecithin 130
 LIGHT, influence on mineral nu-
 trition of swine 494
 LIGHT, photoactivity of cod liv-
 er oil 555
 LIGHT, polarized, influence on
 yeast and bacteria 474
 LIGHT, polarized, influence on
 some convulsant drugs 471
 LIGHT, polarized, influence on
 some ferments 473
 LIVER extracts with blood pres-
 sure reducing properties 483
 LIVER, perfusion studies 367
 LUMINESCENCE, electrical
 stimulation in *Ctenophores* 80
 LUNGS, action of ammonia 199
 LYTIC principle of weak potency 433

MAGNETIC field, constant, ef-
 fect on morphogenesis 125
 MEDIUM, rainbow 522
 MELANURIA in mental disease 135
 MENINGES, experimental study
 of origin 52
 MERCURY combining power of
 deproteinized blood 556
 METABOLISM and respiration
 in starfish, relation to hydro-
 gen ion concentration 54
 METABOLISM in children, in-
 fluence of ultra-violet radiation 431
 METABOLISM of corallines 162
 METABOLISM of dogs under
 amytal anesthesia, effect of
 insulin 424
 METABOLISM of rabbits, in-
 fluence of insulin 66
 METABOLISM of some hetero-
 cyclic compounds 394
 METABOLISM, rate of, and sex
 determination in *Cladocera* 77
 MILK, germicidal action 15
 MONILIA isolation from psori-
 atic patients into human beings 477

MORPHOGENESIS, effect of constant magnetic field	125	PHYTOSTEROL activated by irradiation	319
MUSCLE-EXTRACTIVES, new iminazol-phosphorus compounds	234	PHYTOSTEROL, antirachitic potency	227
MUSCLES, voluntary, sympathetic innervation	23	PITUITRIN, action on coronary arteries of rabbit	507
Experimental observations on functional significance	25	PITUITRIN, effect on blood, lymph, urine	312
NECATOR and Ancylostoma, distinction between larvæ	261	PNEUMOCOCCUS (protective) antibody, precipitation	211
NEOARSPHENAMINE, differentiation from sulpharsphenamine	287	PNEUMOCOCCI cultivation	267
NEOPLASM, transplantable, effect of radiation on malignancy	448	PNEUMOCOCCI treated with sodium ricinoleate, antigenic properties	357
NEPHRITIS in rats on diet deficient in Vitamin A	410	PNEUMOCOCCUS soluble substance, effect in serum leucocyte mixtures	262
NITROGEN, peptide, occurrence in blood	193	POLIOMYELITIS, precipitin reaction	155
NUCLEO-PROTEINS from gram-positive cocci, antigenic relationships	109	PRECIPITIN reaction in epidemic poliomyelitis	155
OTITIS, suppurative, of albino rat	150	PREGNANCY, acid-base balance	513
OVARIAN extracts	106	PREGNANCY, early human diagnosis by vaginal smear method	436
OVARIOTOMY followed by sex-reversal in fowl	28	PRESERVATION of eggs, effect on vitamin contents	263
OXYGEN tension, virulence and growth changes of <i>B. lepi-septicum</i>	139	PROTEIN diets, high, effect on kidneys of rats	482
PANCREAS, insulin content in animals poisoned with phlorhizin	74	PROTEIN in dental enamel	175
PANCREAS, perfusion studies	367	PROTEINS of cereals, alcohol-soluble, immunological study	185
PARAMECIUM, relation of contractile and food vacuoles to rhythms	333	PROTEINS of cotton-seed	226
PARASITES of <i>blastomycosis hominis</i> , ascopore stage	69	PROTEUS bacilli, action of glycerol	399
PARATHYROID glands, experiments with abstracts	447	PROTOPLASM of living amœba, reactions to injected salts	320
PARATHYROID gland, hormone	560	PROTOZOA, intestinal, transfer from man to monkeys	206
<i>PARS tuberalis</i> of the <i>hypophysis cerebri</i> , quantitative studies	499	PURINE bases, micromethod for nephelometric estimation	119
PEPSIN, action on insulin	9	PYLORUS, dehydration following obstruction	365
PERMEABILITY of lecithin, effect of light	130	QUINIDINE, effect on interauricular conduction and irritability	311
PHAGOCYTOSIS, surface tension factor, and observations on bacteria in films	4	RADIATION, effects on calcium	469
PHLORHIZIN, insulin content of pancreas in animals poisoned	74	RADIATION, effect on malignancy of transplantable neoplasm	448
PHOSPHATE, calcium, solubility product	283	RADIATION, effects on phosphorus	469
PHOSPHATES parenterally administered	294	RADIATION, ultra-violet, influence on basal metabolism in children	431
PHOSPHORUS of normal rabbits	315	RADIUMIZED media, effect upon tissue cultures <i>in vitro</i>	464
		RAMON flocculation test for scarlatinal antitoxin	426

- RAMON flocculation test in relation to antigenic value of diphtheria toxoid 454
- REFLEX association of feeding and defecation 295
- REFRACTIVE index of Chitin..... 256
- REGENERATION of cut spinal cord in fish 17
- REGENERATION of optic nerve of tadpole after section 476
- RESPIRATION and blood-reaction 82
- RESPIRATION and continuous recording changes of hydrogen ion concentration in circulating blood 298
- RESPIRATORY changes induced by muscular exercise upon stenosis 64
- RETICULO-ENDOTHELIAL system in relation to antibody formation 1
- RICKETS in rats, effect of double adrenalectomy 103
- S**ALVARSAN, alkalinized, behavior of leucocytes after intravenous administration 95
- SCARLATINÆ antistreptococcus horse serum 189
- SCARLATINAL streptococcus toxin, early and late immunity results 460
- SCARLET fever immunization with sodium ricinoleate 549
- SCARLET fever streptococci, individual reactions to toxins 85
- SCARLET fever streptococci, reactions induced by intracutaneous injections 80
- SCHULTZ-DALE technique with Fleisch-Ringer solution—Ca..... 258
- SCURVY and production and maintenance of intercellular substances 400
- SCURVY, changes in testis of guinea pig 177
- SEA-WATER, carbonic acid-carbonate equilibrium 55
- SECRECTIONS internal hepatic, in canine anaphylaxis 61
- SERA, hemolytic immune, flocculation reactions 170
- SERUM-LEUCOCYTE mixtures, effect of pneumococcus soluble substance 262
- SEX-DETERMINATION and rate of metabolism in Cladocera 77
- SEX in Cladocera, a biochemical substance associated with 466
- SEX in pigeons and blood calcium 222
- SEX ratio in mice classified by prenatal mortality 389
- SEX-REVERSAL following ovariectomy in fowl 28
- SHOCK, effect of ephedrine 203
- SODIUM bicarbonate, effect on the excretion of acetone 70
- SODIUM ricinoleate preparation 553
- SPINAL cord, cut, in fish, regeneration 17
- SPINAL fluid, lactic acid content 322
- SPORE-FORMATION in bacteria 197
- STAPHYLOCOCCUS aureus*, production of exotoxin 14
- STARCH and starch pastes, effect of grinding 60
- STENOSIS, effect on respiratory changes induced by muscular exercise 64
- STREPTOCOCCI treated with sodium ricinoleate, antigenic properties 357
- STREPTOCOCCUS erysipelatis*..... 292
- STREPTOCOCCUS, scarlatinal, reactions in guinea pigs 282
- STREPTOCOCCUS toxin, studies of the formation 467
- STROPHANTHIDIN, emetic action 371
- STROPHANTHUS, action on chloralized heart 530
- SUGAR concentration in blood during insulin action 72
- SULPHARSPHENAMINE, differentiation from neoarsphenamine 287
- SURFACE tension, effect on *L. Bulgaricus* and *L. Acidophilus* 337
- SURFACE tension factor in phagocytosis and observations on bacteria in films 4
- SYMPATHETIC innervation of voluntary muscles 23
- Experimental observations on functional significance 25
- SYPHON, constant dropping, for diluting lipid antigen..... 146
- T**ESTIS of guinea pig, during scurvy and inanition 177
- TETANUS bacilli, immunity..... 562
- TETANY, experimental and diet 49
- THERAPEUTIC agent, distribution through intestinal tract 487
- THYROIDECTOMY, physical-chemical changes in blood 478
- TISSUE fluids of Egyptian cotton-leaf, accumulation of chlorides 415

TETANY	117	VEINS , cyanosis of peripheral engorgement	87
THYROXIN, physiologic action..	307	VERATRIN in sciatic nerve of frog	336
TOXINS, bacterial, effect of sodium ricinoleate	194	VITAL staining of centrifuged marine eggs	305
TOXIN neutralization by sodium ricinoleate	550	VITAMIN A lacking in basal dietary	265
TUBERCULIN, chemical study..	209	VITAMIN A, nephritis in rats on diet deficient	410
TUBERCULIN, phosphatids	346	VITAMIN A, relation to growth, reproduction and longevity	75
TUMORS, carbohydrate metabolism	254	VITAMIN B crystals from Mung bean	228
TYPHUS fever, action of glycerol on virus	399	VITAMINS, biological significance	241
ULTRAVIOLET irradiation, anti-rachitic properties imparted to inert fluids.....	5	VITAMIN contents of preserved eggs	263
Imparted to lettuce and growing wheat	6	VITREOUS humor of animal eyes, chemical composition	445
UREA, gasometric determination with urease	486	WEIGHT curves of castrated kids	248
UREASE, gasometric determination of urea	486	WHEAT, growing, and lettuce, antirachitic properties imparted to by ultraviolet irradiation	6
UREASE, insoluble	504	XEROPHTHALMIA , experimental, epithelial tissues	402
URIC acid, micromethod for nephelometric estimation	119	XEROPHTHALMIA in turkeys..	184
URIC acid excretion in alkalosis	512	X-RAYS, relative susceptibility of eggs and sperm of arbacia....	19
URINE and blood constituents in dog, effect of light	171		
URINE of eunuchs, chemical studies	408		
URINE, use of bicolorimeter for estimation of hydrogen ion concentration	511		

EXECUTIVE PROCEEDINGS

ELECTION OF OFFICERS.

At the annual meeting of the Society on April fifteenth, the following officers were elected for the coming year :

President, James W. Jobling, of Columbia.

Vice-President, Stanley R. Benedict, of Cornell.

Secretary-Treasurer, A. J. Goldforb, of C. C. N. Y.

Councilor, W. W. Palmer, of Presbyterian.

(Peyton Rous, of the Rockefeller, to serve his second year).

Nominating Committee :

C. P. Sherwin, Chairman,

H. C. Sherman,

Victor C. Myers,

Holmes C. Jackson,

Donald D. Van Slyke,

Lafayette B. Mendel,

J. B. Collip.

Board of Editors (to be appointed by the President).

TREASURER'S REPORT

September 1, 1924—September 1, 1925.

RECEIPTS

	To Mar. 31, 1925	Estimated Mar. 31 to Sept. 1, 1925	Estimated TOTAL Sept. 1, 1924, to Sept. 1, 1925
Balance on hand -----	\$ 146.10	\$-----	\$ 146.10
Dues -----	2,447.12	250.00	2,697.12
Excess Space -----	488.75	160.00	648.75
Reprints -----	895.98	298.00	1,193.98
Postage -----	27.74	8.00	35.74
Extras -----	168.72	50.00	218.72
Subscriptions -----	747.89	125.00	872.89
Int. Endowment Fund---	175.10	175.10	350.20
	<hr/>	<hr/>	<hr/>
	\$5,097.40	\$1,066.10	\$6,163.50

DISBURSEMENTS

Publisher -----	\$3,200.58	\$2,080.00	\$5,280.58
Printing -----	213.93	35.00	248.93
Sec. Ass't. -----	493.00	234.00	727.00
Insurance and Storage --	26.00	10.00	36.00
Stationery -----	69.60	---	69.60
Miscellaneous			
Bank Exchange -----	2.70	0.90	3.60
Lancaster Mailing ----	13.92	4.64	18.56
Notary Fees -----	.75	.25	1.00
Addressograph -----	3.52	1.00	4.52
Stamps -----	131.61	25.00	156.61
Back Nos. Bought ----	13.00	10.00	23.00
Refunds -----	1.85	.60	2.45
Ret'd. Cheques -----	9.00	3.00	12.00
	<hr/>	<hr/>	<hr/>
	\$4,179.46	\$2,404.39	\$6,583.85
Net Deficit, Sept. 1, 1924 -----			\$760.83
Estimated Deficit, Sept. 1, 1925 -----			420.35
This means that without a deficit on September 1, of \$760.83,			
there would have been a surplus of ----- 340.48.			

BUDGET

September, 1925—September, 1926.

RECEIPTS	DISBURSEMENTS
Dues ----- \$2,900.00	Publisher ----- \$4,700.00
Excess Space ----- 650.00	Printing ----- 300.00
Reprints ----- 1,300.00	Sec. Assistant ----- 1,300.00
Postage ----- 35.00	Insurance and Storage 36.00
Extras ----- 218.00	Stationery ----- 75.00
Subscriptions ----- 900.00	Miscellaneous
Int. on Endow't Fund 375.00	Bank Exchange -- 4.00
	Lancaster Mailing_ 15.00
\$6,428.00	Notary Fees ----- 5.00
	Addressograph --- 5.00
	Stamps ----- 160.00
	Back Nos. Bought_ 50.00
	Addressograph ----- 150.00
	Office Equipment --- 50.00
	Deficit 1924-1925 --- 420.00
	Branch Rebates ---- 35.00
	Telegrams ----- 10.00
	\$7,315.00

REPORT ON ENDOWMENT FUND

In Railroad Co-op. Build'g & Loan Ass'n, Sept. 1, 1924	\$7,049.66
Received from Members, Sept. 1, 1924, to April 1, 1925	837.00
Total -----	\$7,886.66

CIRCULATION REPORT

April 1, 1925.

Members -----	692
Subscribers -----	134
Honorary Members ----	3
Free Subscriptions ----	47
Exchange List -----	9
Total -----	885

From October first to date, there were :

Resignations -----	9
New Members -----	74
Subscribers, October 1, 1924-----	61
Subscribers, April 1, 1925-----	134

COST TO AUTHORS

Average Cost	
Excess Space -----	\$3.30
Reprints -----	5.66
Extra Cuts -----	1.75
Average Total Cost -----	\$10.71
Average Number of Reprints -----	210

The President was authorized to appoint a committee to revise the constitution, with particular reference to the closer relation of the Branches to the Society. The following were appointed: Wm. J. Gies, (Chairman), H. C. Sherman, (Columbia), I. Greenwald, (Roosevelt Hospital), G. M. Mackenzie (Presbyterian), H. C. Jackson (Bellevue Medical College), and the Secretary.

The President was authorized to appoint a committee to take the necessary steps to incorporate the Society: Wm. J. Gies (Chairman).

The Council decided to postpone carrying advertising in the PROCEEDINGS, but have the President appoint a Committee to investigate the possibility of obtaining a substantial increase to the Endowment Fund, to meet the deficit. There was, in spite of the increase in office expenses, a decrease in the deficit. With increase in the Endowment Fund and continued economies, it may be possible to meet the deficit without increasing cost to members or authors.

New Branches were established: Southern Branch (New Orleans), Iowa Branch, Missouri Branch (St. Louis), Massachusetts Branch (Boston), Illinois Branch (Chicago).

The Council approved granting 5 per cent of Branch dues toward local expenses. It approved the election in Branches of "Associates" who are not eligible to membership. These Associates may receive the PROCEEDINGS at the same rate as members.

The Council is anxious to ascertain how many of the last year's and the current year's contributions to the PROCEEDINGS are completed reports, and have not or will not immediately be published in extenso elsewhere. It desires this information in order to bring the data before the A. M. A. and other abstract journals, so that the PROCEEDINGS may also be abstracted. Will you take the trouble to notify the Secretary which of your contributions belong in this category.

E. V. McCOLLUM